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Understanding the texture of cooked rice from the molecular, instrumental and sensory levels

Hongyan Li

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Abstract

In recent years, consumer preferences have shifted towards better-quality rice, particularly towards varieties with good eating quality. Texture is an extremely important attribute for cooked rice and has been used as an indicator for consumer acceptance. Cooked rice texture is affected by a wide range of factors, such as the amylose content, postharvest processing, the milling ratio, the cooking method, etc., but the actual molecular reasons for the texture of cooked rice grains are still unclear. Since texture has been defined as a multidimensional characteristic that only humans can perceive, define, and measure, sensory descriptive analysis is a useful tool for characterizing texture properties of cooked rice. However, the cost associated with training and maintaining a descriptive panel has prompted many researchers to evaluate less costly and less time-consuming approaches. The overall objectives of this thesis are to explore the molecular mechanisms for the hardness and stickiness of cooked rice grains, increase understanding of the human textural perception of cooked rice, and develop an improved instrumental method to evaluate and/or predict the texture of cooked rice.

The first chapter of this thesis reviews current understanding of the texture of cooked rice, which involves the factors affecting rice texture, the evaluation methods for cooked rice texture, and the scientific questions generating from the literature review and associating to the overall objectives of this thesis.

In chapter 2, statistically and causally meaningful relationships are established between starch molecular structure (the molecular size distribution of whole (branched) starch and the chain length distribution of debranched starch) and texture (hardness and stickiness) of cooked rice grains. The amounts of amylose chains with degree of polymerization (DP) 100-20000, and of long amylopectin chains, positively correlate with hardness, while amylopectin chains with DP<70 and amylose molecular size both show negative correlations with hardness (p<0.05). There is also a significant negative correlation between stickiness and the amounts of long amylopectin chains (p<0.01). For rices with similar amylose content, the amount of amylose chains with DP 1000-2000 positively correlates with hardness while size negatively correlates with hardness (p<0.05). This indicates for the first time that, regardless of amylose content, rice varieties with smaller amylose molecular sizes and with higher proportions of amylose chains with DP 1000-2000 have a harder texture after cooking. This can be rationalized in terms of viscosity effects of long chains.

Chapter 3 presents the first molecular understanding of stickiness between cooked rice grains by measuring the leaching and molecular structural characteristics during rice cooking. We find (i) the molecular size of leached amylopectin is 30 times smaller than that of native amylopectin while (ii) that of leached amylose is 5 times smaller than that of native amylose, (iii) the chain-length distribution (CLD: the number of monomer units in a chain on the branched polymer) of leached amylopectin is similar to native amylopectin while (iv) the CLD of leached amylose is much narrower than that of the native amylose), and (v) mainly amylopectin, not amylose, leaches out of the granule and rice kernel during cooking. Stickiness is found to increase with decreasing amylose content in the whole grain, and, in the leachate, with increasing total amount of amylopectin, the proportion of short amylopectin chains, and amylopectin molecular size. A molecular adhesion mechanism is put forward to explain this result. This molecular structural mechanism provides a new tool for rice breeders to select cultivars with desirable palatability by quantifying the components and molecular structure of leached starch.

Chapter 4 characterizes the cooked rice texture by descriptive sensory analysis and two instrumental methods (texture profile analysis (TPA) and dynamic rheological testing) using a set of 18 varieties of rice with a wide range in amylose content (0-30%). Panellists' results indicate that hardness and stickiness are the two most discriminating attributes among 13 tested textural attributes. Consistency coefficient (K^*) and loss tangent (tan δ) from dynamic frequency sweep are used to compare with hardness and stickiness tested by TPA and sensory panellists, showing that K^* representing hardness and tan δ representing stickiness are both statistically and mechanistically meaningful. The instrumental method is rationalized in terms of starch structural differences between rices: a higher proportion of both amylose and long amylopectin branches with DP 70–100 causes a more elastic and less viscous texture, which is readily understood in terms of polymer dynamics in solution.

Finally, conclusions are presented in Chapter 5, summarizing the mechanisms for the hardness and stickiness of cooked rice, the main achievements corresponding to the objectives of this thesis, and the potential application of this study for rice industry and rice breeders. Furthermore, future works, e.g. exploring the specific location of amylose molecules within starch granules, optimizing the reference samples for sensory training, learning the effect of mastication and saliva on the rheological properties of cooked rice, are also recommended.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Journal Publications Relevant to Thesis

1. **Li, H.,** Fitzgerald, M. A., Prakash, S., Nicholson, T. M., & Gilbert, R. G. (2017). The molecular structural features controlling stickiness in cooked rice, a major palatability determinant. *Scientific Reports, 7*, 43713.
2. **Li, H.,** Prakash, S., Nicholson, T. M., Fitzgerald, M. A., & Gilbert, R. G. (2016a). The importance of amylose and amylopectin fine structure for textural properties of cooked rice grains. *Food Chemistry, 196*, 702-711.
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Contributor	Statement of contribution
Li (Candidate)	Designed and carried out experiments (100 %) Data analysis (100 %) Wrote the paper (90 %)
Dr Prakash	Review the paper (10 %)
Dr Nicholson	Review the paper (20 %)
Prof. Fitzgerald	Wrote the paper (5%) Review the paper (30 %)
Prof. Gilbert	Wrote the paper (5%) Review the paper (40 %)

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Li (Candidate)	Designed and carried out experiments (100 %) Data analysis (100 %) Wrote the paper (95 %)
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Dr Nicholson	Data analysis (10%) Review the paper (30 %)
Prof. Fitzgerald	Review the paper (20 %)
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Keywords

Rice, texture, mouthfeel, starch, amylose, amylopectin, chain-length distribution, molecular size, hardness, stickiness

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List of abbreviations

AGPase	Single nucleotide polymorphisms
Am	Amylose
ANOVA	Analysis of variance
Ap	Amylopectin
ASV	Alkali spreading value
AUC	Area under the curve
CLD	Chain-length distribution
DB	Degree of branching
DBE	Starch debranching enzyme
DMSO	Dimethyl sulfoxide
DOM	Degree of milling
DP	Degree of polymerization
FACE	Fluorophore-assisted carbohydrate electrophoresis
G'	Storage or elastic modulus
G''	Loss or viscous modulus
GBSS	Granule-bound starch synthase
GPC	Gel-permeation chromatography
GT	Gelatinization temperature
HHP	High hydrostatic pressure
HPAEC	High-performance anionic-exchange chromatography
K^*	Consistency coefficient
LVR	Linear viscoelastic region
$N_{de}(X)$	Number chain-length distribution

NIR	Near-infrared spectroscopy
PCA	Principal component analysis
QDA	Quantitative descriptive analysis
R_h	Hydrodynamic radius
SBE	Starch branching enzyme
SD	Standard deviation
SEC	Size-exclusion chromatography
-SH	-Sulfhydryl
SNP	Single nucleotide polymorphisms
-SS-	-Disulfide
SS	Starch synthase
tan δ	Loss tangent
TPA	Texture profile analysis
V_h	Hydrodynamic volume
$w(\log X)$	Weight chain-length distribution

Chapter 1 Literature review on the texture of cooked rice grains

1.1 Introduction

Rice is the most important staple food for human consumption. More than 90% of it is grown and consumed in South, East, and Southeast Asia, where ~60% of the earth's population lives (Bhattacharya, 2009). With the high levels of economic growth in Asia over the past thirty years lifting millions of people out of poverty, producing a higher proportion of middle class people, the demand for higher quality rice is rapidly increasing (Lee & Hong, 2012). However, rice consumers, particularly from countries for which rice is the staple, have strong preferences for the sensory properties of rice. Different countries have different requirements for quality, and within countries, a range of preferences can found (Champagne et al., 2010). Thus, one of emerging challenges facing the rice industry and breeders is to control the eating quality of rice for specific end-use markets. Cooked rice texture has been shown to govern the acceptance of rice by consumers (Okabe, 1979).

Of all of the major cereals, rice is the only one that is consumed mostly in the form of whole grains after cooking. Cooked rice texture is affected by a wide range of factors, such as the amylose content (Juliano, Onate & Del Mundo, 1972), postharvest processing (Champagne et al., 1998), the milling ratio (Lyon et al., 1999; Park, Kim & Kim, 2001), and the cooking method (Leelayuthsoontorn & Thipayarat, 2006). Since texture has been defined as a multidimensional characteristic that only humans can perceive, define, and measure (Szczesniak, 1987), sensory descriptive analysis has been shown to be a useful tool for characterizing the textural properties of cooked rice. However, the cost associated with training and maintaining a descriptive panel has prompted many researchers to evaluate less costly and less time-consuming approaches (Champagne et al., 1999; Meullenet, Champagne, Bett, McClung & Kauffmann, 2000; Sesmat & Meullenet, 2001).

Therefore, it would extremely beneficial to learn the main factors determining the cooked rice texture, and to develop more advanced techniques to evaluate rice eating quality for rice breeders and industry. This review will present the current perspectives on two aspects: 1) Factors affecting rice texture, 2) Evaluation methods for the cooked rice texture. The developments in each of these subjects are reviewed below.

1.2 Rice composition

The rice grain (**Fig. 1.1**) comprises the hull (16-28% dry mass basis) and the caryopsis. Removal of the hull during milling produces brown rice. The mass distribution of the rice caryopsis is: pericarp, 1-2%; aleurone plus seed coat and nucellus, 4-6%; embryo, 2-3%; and starchy endosperm, 89-94%. The aleurone layer is from one to five cell layers and is thicker at the dorsal side compared to the ventral side. This layer is also thicker in short-grain compared to long-grain rice (Delrosar, Briones, Vidal & Juliano, 1968). Further milling,, that removes the pericarp, seed coat, testa, aleurone layer and embryo, yields milled or white rice; this results in a disproportionate loss of lipid, protein, fibre, reducing sugars and total sugars, ash and minor components including vitamins, free amino acids and free fatty acids (Park, Kim & Kim, 2001). Diastatic, proteolytic and lipolytic activities are also reduced by milling. On the other hand, available carbohydrates, mainly starch, are at a higher percentage in milled rice compared brown rice. Starch is the major constituent of milled rice, making up ~90% of the dry matter. Protein and lipid contents are also significant. The endosperm cells are thin-walled and packed with amyloplasts containing compound starch granules which are evenly distributed, although they are smaller in size near the periphery of the endosperm. The two outer-most cell layers (the subaleurone layer) are rich in protein and lipids, and have smaller amyloplasts and compound starch granules than the inner endosperm (Zhou, Robards, Helliwell & Blanchard, 2002).

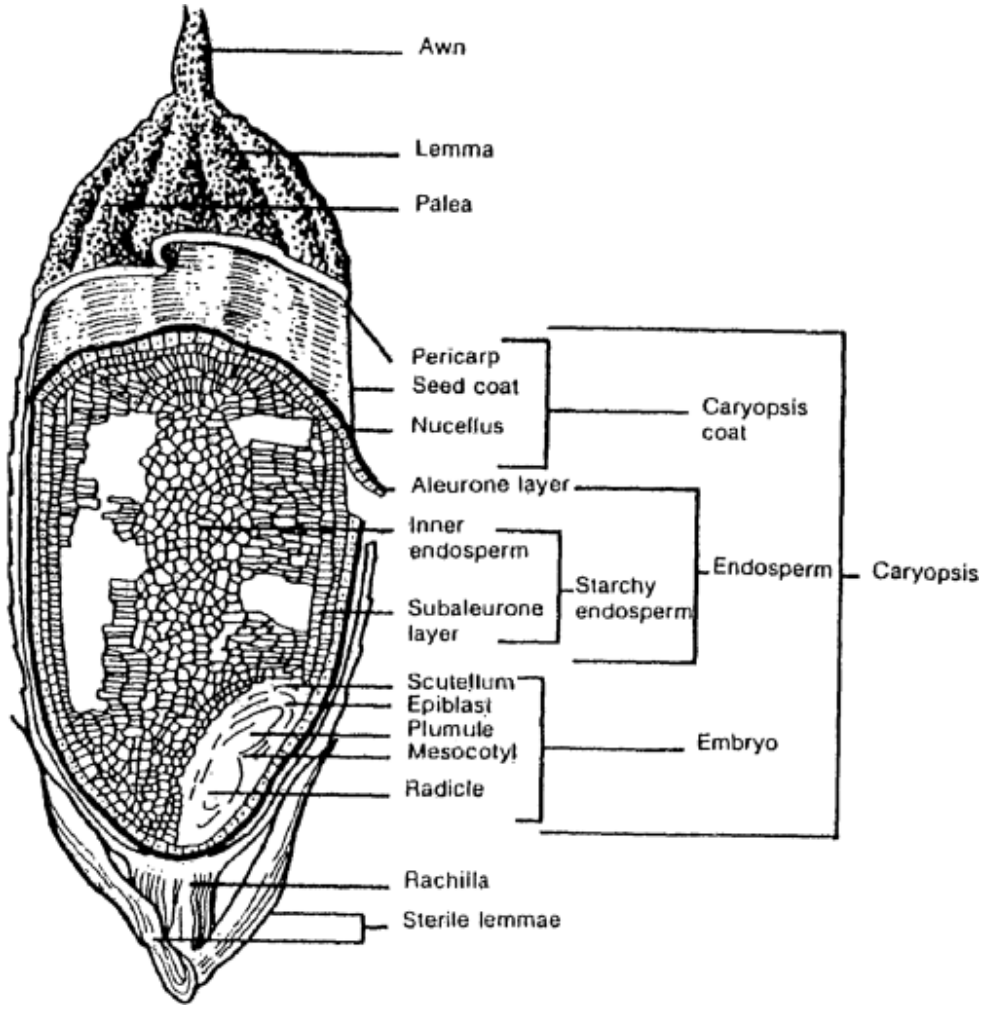

Figure 1.1 The detailed structure of rice grains (Zhou, Robards, Helliwell & Blanchard, 2002).

1.3 Factors affecting rice texture

1.3.1 Starch

Starch is a branched polymer comprised of chains of D-glucose units connected via α-(1→4) linkages and branch points consisting of α-(1→6) linkages. It comprises two types of molecules: amylopectin and amylose. Amylopectin molecules are highly branched glucose polymers with a vast number of short branches and high molecular weights, whereas amylose molecules have smaller molecular weights with little branching but long chains. Starch's structure is complex but can be divided into multiple structural levels, as illustrated in **Fig. 1.2**. Level 1 describes that of the individual chains, with data normally presented as the chain-length distribution, which is the relative number of chains as a function of their degree of polymerization. These CLDs comprise the short chains in amylopectin, with average chain lengths (or degree of polymerization, DP) of 17-25 and the long chains in amylose, having

average DPs typically between 10^3 and 10^4. Level 2 is that of the fully branched individual starch molecules. Those molecules comprise amylose, with molecular weights of ~10^6, and the highly branched amylopectin, with a typical molecular weight approximately two orders of magnitude greater than amylose. Level 3 describes the conformation of starch molecules, encompassing the following features: starch chains aggregate, entwining into a helical structure; the helices aggregate to form crystallites; and, finally, the crystallites form alternating amorphous and crystalline lamella. The double helices form A-type polymorphic crystallites with a monoclinic unit cell for rice. Starch, especially amylose, can also form single-helical complexes with some lipids, alcohols, or ions, in which the complex molecule occupies the central cavity of the helix, forming V-type crystallites. The crystalline portion of the lamellar structure in native starch granules is composed largely of amylopectin chains, with those chains with DPs of 12-24 promoting the production of the most stable crystalline structures. The branch points of amylopectin, along with some portions of the chains, are mostly located in the amorphous lamellae. The average repeat distance of the combined amorphous-crystalline lamellae in native starch granules is ~9-10 nm. Higher structural levels of native starch granules comprise growth rings and individual granules that are associated with non-starch components such as non-starch polysaccharides, proteins, and lipids (Gilbert, Witt & Hasjim, 2013).

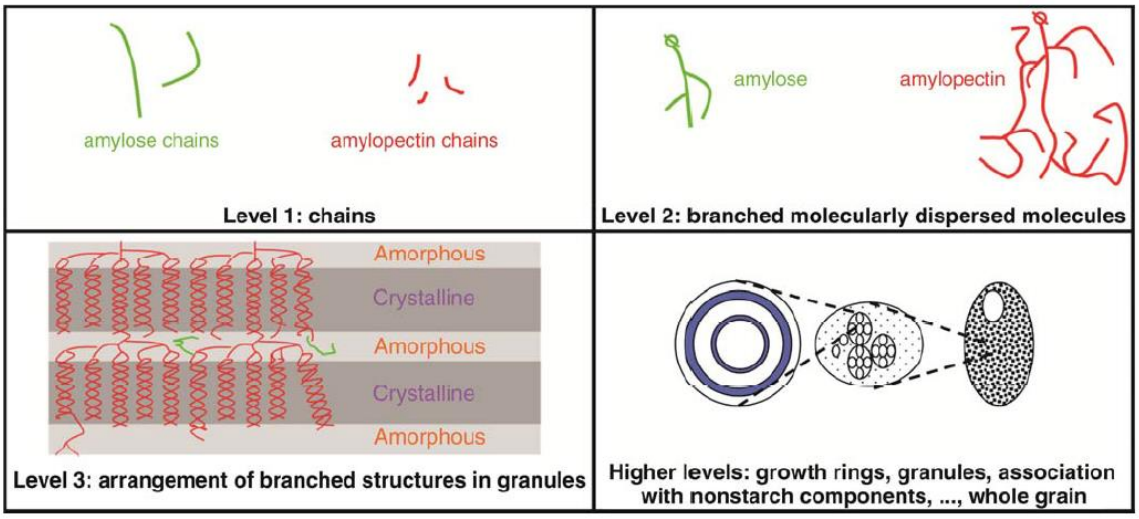

Figure 1.2 The structural levels of starch (Gilbert, Witt & Hasjim, 2013).

Because starch accounts for up to 90% of the dry matter in a milled rice grain, it is reasonable to focus on starch's contribution to rice texture. Since the 1950s, Sanjiva Rao and his associates first suggested a possible relation between the amount of amylose, as determined

by iodine colorimetry, and rice quality (Rao, Murthy & Subrahmanya, 1952). Later amylose was found to correlate well with rice texture (Juliano, Onate & Delmundo, 1965; Williams, Wu, Tsai & Bates, 1958). A positive relation between the amylose content and sensory or instrumental values of hardness and an inverse relation with the stickiness of cooked rice was reported, with this view being generally accepted for three and a half decades, until the mid-eighties (Hampel, 1965; Juliano, 1984; Juliano et al., 1981; Kumar, Upadhyay & Bhattacharya, 1976; Lorenz, Fong, Mossman & Saunders, 1978). However, it was also recognized that the content of apparent amylose was a necessary but not a sufficient factor affecting cooked rice texture, since varieties with similar or identical apparent amylose often displayed differences in quality due to certain unknown secondary factors, the understanding of which was the major quest of rice chemists during the previous two to three decades. A variety of secondary factors were suggested, such as the alkali spreading value (ASV), the gel consistency, the amylograph viscosity profile, and the pasting pattern (Cagampang, Perez & Juliano, 1973; Juliano, Villareal, Perez, Villareal, Takeda & Hizukuri, 1987; Perez, Villareal, Juliano & Biliaderis, 1993). Later, Bhattacharya, Sowbhagya and Swamy (1982) showed that the hot water (96 ℃) solubility of apparent amylose differed among varieties. The high-amylose rice varieties in particular fell into three distinct groups, with apparent amylose solubilities being approximately 40%, 50%, and 60%; these three groups of rice differed distinctly in all of their physicochemical and textural attributes. Based on these results, a new parameter termed ‘hot-water-insoluble amylose’ (total amylose minus the soluble amylose) was proposed as a key determinant of rice quality, as this correlated well with texture, and other physicochemical parameters of rice (Sandhya Rani & Bhattacharya, 1985). Further, a dramatic shift of focus arose after the mid-eighties. Chinnaswamy and Bhattacharya (1986) separated rice starch by gel-permeation chromatography over Sepharose 2B and noted that the high-molecular-weight branched fraction (FRI) of starch, in terms of its iodine reaction, correlated well with the insoluble amylose of rice. The low-molecular-weight fraction (FRII) seemed to correlate with the soluble amylose, which was apparently texturally unimportant. These findings were later confirmed by Radhika Reddy, Zakiuddin Ali and Bhattacharya (1993). Meanwhile, Takeda et al. (1987, 1989) and Hizukuri et al. (1989) observed that chemically isolated rice amylopectin, upon debranching, yielded three groups of anhydro-glucose chain populations (long-B, intermediate-B and A plus short-B). High-amylose rice had more long-B chains than low-amylose rices (Hizukuri, Takeda, Maruta & Juliano, 1989; Takeda, Hizukuri & Juliano, 1987; Takeda, Maruta, Hizukuri & Juliano, 1989). Radhika

Reddy, Zakiuddin Ali and Bhattacharya (1993) isolated the GPC-separated FRI (amylopectin) of several rice varieties of various amylose contents and studied their chain profile. They found that the proportion of long-B chains, as well as their external chains, in the molecule was strongly and positively correlated with the insoluble amylose contents of the parent rice varieties. On this basis, it was believed that the content and disposition of the long-B chains of amylopectin was the key determinant of rice texture. The FRII was assumed by the authors to consist primarily of the soluble amylose, and hence suggested that the true amylose content does not affect rice quality. On the basis of their own study and other parallel rheological and microscopical studies, they concluded that the long B chains by virtue of intermolecular interactions rendered the starch granule strong and resilient, thus leading to the firm texture of the cooked rice. A shortage of these chains led to weak starch granules and hence, leading in turn to soft cooked rice. Furthermore, Ong and Blanshard (1995a) confirmed that rice varieties with a greater number of long chains in the amylopectin resulted in hard-cooking parboiled rice. Interestingly, all of these molecular findings were from the same institute, the Central Food Technological Research Institute (CFTRI) in India. The differences in the strength of the starch granules caused by the relative abundance of long chains in their starch molecules thus seemed to be at the root of the differences in rice texture. Furthermore, in most of the above studies the stickiness was negatively correlated with the hardness or firmness. It appears that stickiness is attributed to amylopectin, especially the short chains (A and short B chains) of amylopectin (Cameron & Wang, 2005; Chinnaswamy & Bhattacharya, 1986; Hizukuri, Takeda, Maruta & Juliano, 1989; Ong & Blanshard, 1995a). However, for some rice varieties, even though their amylose contents are comparable, their stickiness differs significantly. Ayabe, Kasai, Ohishi and Hatae (2009) investigated the stickiness of cooked Nipponbare and Khao Dawk Mali rice, which have similar amylose contents, and attributed differences of stickiness to the solid content and the amount of amylopectin from the surface of cooked rice grains. They suggested that the stickiness of cooked rice is less when less amylopectin is dissolved into the cooking water, even when the amylose content and fine structure of the starch in the rice grains are similar.

1.3.2 Protein

Rice protein, which accounts for 7-8% (Dry Basis, db) of milled rice kernels, is classified into four types: alkali-soluble glutelins (80%), water-soluble albumins (9-11%), salt-soluble globulins (7-15%), and alcohol-soluble prolamins (2-4%) (Landers & Hamaker, 1994).

Among those, albumin and globulin existing in the aleurone layer are usually removed during milling. Heterogeneous large molecules of glutelins exist inside the rice endosperm in the forms of protein bodies (Juliano & Boulter, 1976). These spherically shaped protein bodies bind strongly to the compound starch granules with strong disulfide bonds and/or hydrophobic bonds (Tanaka, Resurreccion, Juliano & Bechtel, 1978).

There are conflicting reports regarding the possible role of the protein content on the sensory and processing qualities of rice. It was postulated that a high protein content in the outer layers of rice causes a reduction in stickiness after cooking (Primo, Casas, Barber & Benedito de Barber, 1962). A close relationship between eating quality and sulfhydryl (-SH), and in particular disulfide (-SS-) groups, was observed (Primo, Barber & Benedito de Barber, 1965). Sensory evaluation initially showed that cooked rice with relatively higher protein levels was significantly less tender than rice with low protein contents (Onate, Del Mundo & Juliano, 1964). The extensive studies of Juliano, Onate and Del Mundo (1972), including isogenic lines differing only in the protein content, did not find protein to have a significant effect on the sensory scores of cooked rice. Later, in more detailed studies at IRRI on world rice samples, an international cooperative test of instrumental texture measurement (Juliano et al., 1981) and studies at CFTRI (Bhattacharya & Sowbhagya, 1972; Bhattacharya, Sowbhagya & Swamy, 1982), protein content was not found to have any effects on rice quality.

This view is now modified by more recent research. Yanase, Ohtsubo, Hashimoto, Sato and Teranishi (1984) suggested that the protein content of rice was inversely related to its viscographic breakdown and cooked rice adhesiveness. Hamaker and Griffin (1993) showed that the Brabender viscogram of rice flour was lowered when the slurry was treated with a reducing agent to break the -SS- bonds. Simultaneously, the stickiness of cooked rice also decreased. Hamaker (1993) has reviewed other circumstantial evidence that suggests that protein may play a role in rice quality. Chrastil (1993) suggested that the rice protein oryzenin plays a major role in the changes brought about in rice texture during aging. Okadome, Toyoshima and Ohtsubo (1999) provided evidence that while the overall hardness of cooked rice is mostly determined by its starch, its surface hardness is related more to the protein content. Kim, Hong, Kim, Lee and Park (1997) have shown with a Korean rice that the protein content is negatively correlated with the rice's palatability. Martin and Fitzgerald (2002) suggested that the protein content affects the amount of water that rice absorbs early in cooking, and the availability of water during the early stages of cooking will determine the

hydration of the protein and the concentration of the dispersed and viscous phases of starch, which will affect the texture of cooked rice. Derycke, Veraverbeke, Vandeputte, Man, Hoseney and Delcour (2005) explored the impact of proteins on pasting and cooking properties of nonparboiled and parboiled rice, and conclude that protein can act as a barrier affecting starch swelling, rheological and cooking properties of both nonparboilded and parboiled rice.

1.3.3 Postharvest processing conditions

Since rice is usually used and consumed as milled whole rice after removing the outer hull and the bran layers of the rough rice, it is important to know the effects of postharvest processing including the drying conditions, the final moisture content and the degree of milling (DOM) on the eating quality of rice. Drying conditions differ between countries and regions; for example in the U.S. rice is mechanically dried, while in Japan ~10% is window-dried on racks in the field with the remainder being mechanically dried. Also rough rice is commonly dried to a 12% moisture content in the U.S., in contrast to the 14-15% content in Japan (Champagne et al., 1998). Champagne et al. (1998) reported that the instrumentally measured textural properties were not significantly affected by the drying conditions, with the exception of cohesiveness. Lyon et al. (1999) also reported a similar conclusion, that drying conditions did not significantly affect the textural properties of cooked rice.

The rice moisture content at harvest, an indicator of rice kernel development, is another important factor that affects rice quality (Wang, Siebenmorgen, Matsler & Bautista, 2004). For instance, the draining and harvesting date, which influences the rice moisture content at harvest, has been reported to affect rice metabolic processes and starch and protein composition as well as their structure (Champagne, Bett-Garber, Thompson, Mutters, Grimm & McClung, 2005). Although starch and protein synthesis is thought to be complete when the rice moisture content reaches 27-29%, a slight decrease in rice proteins and lipids and an increase in the amylose content has been reported (Wang, Siebenmorgen, Matsler & Bautista, 2004). This could be because bulk rice at 27-29% still contains significant proportion of immature kernels. Because small variations in rice chemical composition may result in changes in rice physicochemical properties (Chrastil, 1990), variations of the moisture content at the time of rice harvest are expected to lead to variations in the functional properties of cooked rice, including its texture.

The degree of milling refers to how much bran is removed during the milling process (Perdon, Siebenmorgen, Mauromoustakos, Griffin & Johnson, 2001). However, the amount of bran removed varies depending on the dehulling and milling process. Therefore, variations in the DOM results in changes in the rice kernel's gross composition. For example, Juliano et al. (1984) indicated that >98% of the surface lipid content and >50% of proteins are usually removed by milling. The removal of the outer layers of rough rice, due to milling, was reported to cause a disproportionate loss of lipids, proteins, reducing sugars, and minor components, thereby increasing the relative amount of starch in milled rice (Park, Kim & Kim, 2001). Furthermore, the effect of the DOM on the eating quality of cooked rice has been reported elsewhere, with the brown rice being reported as inferior to that of milled rice (Juliano, 1985). However, the increased milling of rice does not always result in a higher eating quality (Yong-Woong & Jeon-Woo, 1991), with reports that increased milling did not improve the sensory quality of cooked rice after a long period of grain storage.Champagne et al. (1998) investigated the effects of drying conditions, the final moisture content, and the DOM on the texture of cooked rice varieties, as measured by texture profile analysis. The effects of deep milling were more pronounced in the rice dried to a 15% moisture content than that dried to 12%. In general, the textural property values for hardness were higher and the values for cohesiveness, adhesiveness, and springiness were lower in regular-milled rice dried to a 15% moisture content than in those dried to 12%. Park et al. (2001) used a quantitative descriptive analysis of cooked rice to investigate the effect of the DOM on the sensory characteristics of cooked rice. A trained panel found that the colour, intactness of grains, puffed-corn flavour, raw-rice flavour, wet-cardboard flavour, hay-like flavour and bitter taste decreased with increased milling, while glossiness, plumpness, and sweet taste increased. The degree of agglomeration, adhesiveness, cohesiveness of mass, inner moisture, and toothpacking of cooked rice increased while the hardness and chewiness decreased with increased milling.

1.3.4 Cooking methods

Unlike wheat, corn or oats, which are milled into flours or rolled before cooking, rice is generally cooked and consumed whole (Marshall, 1993). People in different countries or regions have different cooking protocols. Some cook rice in rice cookers, via the following protocol: milled rice is washed with cold water followed by straining to remove excess water; after washing, rice is transferred to the rice cooker and water is added to give the appropriate

water to rice ratio. Some cook rice using a pan with excess water, using the following method: rice is washed, added to three portions of water (w/w), soaked, and boiled. Another method to cook rice involves complete evaporation (Champagne et al., 2010). It has been observed that most cooking methods are subtle variations of two basic techniques: (i) cooking in large amounts of water, with subsequent drainage (and sometimes rinsing) - commonly referred to as the Excess or American method; or (ii) cooking of rinsed rice in a measured amount (often twice the volume of rice) of water which is absorbed into the rice - commonly known as the Pilaf or Oriental method (Sinki, 1994).

Furthermore, there are some emerging technologies involved in rice cooking, such as microwave heating and high hydrostatic pressure (HHP). In traditional rice cooking, the energy is transmitted from outside to inside, while in microwave heating, the energy is transmitted from the centre to the edge (Li, Han, Xu, Xiong & Zhao, 2014). It was also reported that a panel had equal preference for microwave-cooked rice varieties and rice cooked via the more traditional methods (Khatoon & Prakash, 2007). HHP has been investigated as an alternative to the traditional thermal processing of foods (Hayashi, 1991). In HHP processing, applied pressure is instantaneously and uniformly distributed within the product, removing the influence of sample size on processing times. In addition, HHP processing results in significant energy savings in comparison to thermal techniques, because once the desired pressure is reached, it can be maintained without the need for further energy input (Estrada-Girón, Swanson & Barbosa-Canovas, 2005). Boluda-Aguilar, Taboada-Rodríguez, López-Gómez, Marín-Iniesta and Barbosa-Cánovas (2013) used HHP to cook rice rapidly, and found that rice which was processed with a single HHP treatment at 300 and 400 MPa had a better acceptance than that of other treatments, in terms of grain shape, adhesiveness, cohesiveness, and texture.

Parboiling of rice is an ancient process that originated in India and is still practiced widely in the south Asian region. It is estimated that about 20% of the world's production of paddy rice is parboiled (Delcour & Hoseney, 2010). The process of parboiling involves: 1) steeping the paddy in water until it is saturated (~30% moisture), 2) draining the water, 3) steaming or heating the soaked paddy to gelatinize the starch, and 4) drying the wet grains to the normal moisture content. Parboiling has a pronounced effect on the physical properties (shape, colour, hardness), flow and storage properties, chemical and nutritional properties, physicochemical properties, as well as the cooking and eating quality. For example, parboiled rice takes a

longer time to cook than raw rice; the cooked parboiled rice is firmer than the cooked raw rice (Ramesh, Bhattacharya & Mitchell, 2000). It is suggested that the degradation of the structure of the starch granules during the heat treatment and starch retrogradation during the drying stage of the parboiling process are responsible for reduced starch swelling in parboiled rice, thereby causing a reduced stickiness of cooked rice (Damir, 1985).

Among all these cooking methods, the amount of water added to the grain is one of the major factors that influence cooked rice texture (Bett-Garber, Champagne, Ingram & McClung, 2007; Juliano & Perez, 1983). Long-grain cultivars tend to require more water than medium-grain and short-grain types (Khan & Ali, 1985). If a specific rice cultivar is cooked with a higher water-to-rice ratio than recommended, the resulting cooked rice will have a higher moisture content, be less hard and more adhesive, have a larger grain size, and be less yellow (Juliano et al., 1984). Grain stickiness increases when rice is cooked with increasing water to rice ratios (Kim & Kim, 1996). However when using the excess water method, the water content and stickiness were not related (Juliano et al., 1984). Kim, Kim and Kim (1986) found, using subjective means, that changes in water to rice ratios had an effect on the texture and appearance; however the flavour remained unchanged for the four rice cultivars. Srisawas and Jindal (2007) also examined the effect of the water-to-rice ratio on sensory hardness, stickiness and fragrance. With increasing water-to-rice ratios, the sensory hardness decreased and the stickiness increased, while the fragrance was not significantly affected. Using the amylose content as the criterion to produce appropriate water to rice ratios has been widely accepted and used (Champagne et al., 1999; Meullenet, Champagne, Bett, McClung & Kauffmann, 2000).

1.4 Evaluation methods for cooked rice texture

1.4.1 Physicochemical characteristics as indicators of rice texture

Grain physicochemical properties have been used as indicators of textural properties of rice for a long time. The earliest parameter developed for rice quality studies was most likely the starch-iodine blue value. A warm-water extract of parboiled rice flour gave a deeper blue colour with iodine when compared with the extract of the corresponding raw rice flour (Roberts, Potter, Kester & Keneaster, 1954), and the intensities of the blue colour differed among extracts from different varieties of raw rice (Halick & Keneaster, 1956). Subsequently, the starch-iodine blue value was related to the viscogram pattern and the amylose content

(Batcher, Helmintoller & Dawson, 1956). This test was even extended to the excess water remaining after cooking rice, and the value was thought to correlate with other properties (Hogan & Planck, 1958). However, in a detailed study it was shown that the blue value of the excess cooking water of rice gave no useful information (Bhattacharya, Sowbhagya & Swamy, 1972).

The iodine-blue value was considered to be a reasonable index of the amylose content of rice (Juliano, 1964). However, Juliano and his co-workers observed that the amylose content and iodine-blue value did not correlate well in varieties that had amylose contents above 30% (Juliano, Cartano & Vidal, 1968). Subsequently, Bhattacharya and co-workers reasoned that the blue value actually just measured the dissolved amylose. Based on this, they proposed the new index of "hot-water-insoluble amylose" (determined after dispersion of rice flour in dilute alkali) and soluble amylose (determined by extracting rice flour with hot water). This index showed excellent correlation with textural properties as well as various other properties of high-amylose rice (Bhattacharya & Sowbhagya, 1978).

The Brabender viscogram of rice flour showed a lower peak viscosity and positive setback for high amylose varieties, while showing a higher peak viscosity, higher breakdown and lower setback for sticky rice (El-Saied, El-Attar, Ahmed & Roushdi, 1979; Halick & Kelly, 1959). In addition, the textural differences in rices with similar amylose contents could often be distinguished by the Brabender viscograph criteria (Merca & Juliano, 1981). The sensitivity of Branbender viscography was improved and a new parameter, the 'relative breakdown' (BDr), was introduced, showing very good correlation with rice texture. It was shown that the correlation was much better than with the peak viscosity and setback previously used as viscography indices (Bhattacharya & Sowbhagya, 1978). This parameter also correlated well with insoluble amylose and helped to classify rice varieties into different types, each type having distinct quality characteristics (Sowbhagya, Ramesh & Bhattacharya, 1987).

Since the 1910s, some researchers observed that rice grains could be disintegrated by dilute alkali (Warth & Darabsett, 1914). The extent of disintegration was directly proportional to the concentration of KOH but was depended on the variety. This phenomenon was studied in detail, and it was suggested that the susceptibility of rice kernels to degrade by alkali was a good index of rice texture (Little, Hilder & Dawson, 1958). This test is routinely conducted in the U.S. in screening varieties and also in studies on rice quality (Webb, 1975). Subsequent

work showed that the susceptibility to alkali disintegration correlated inversely with the gelatinization temperature (GT) of rice (Juliano, Bautista, Lugay & Reyes, 1964); however this parameter did not seem to correlate with rice texture for varieties other than waxy rices (Bhattacharya, Sowbhagya & Swamy, 1982). Scientists at CFTRI noted that in addition to the extent of grain disintegration, the pattern or type of degradation also varied among varieties. Five distinct types of disintegration were observed, designated A, B, B_1, C, and D (Bhattacharya & Sowbhagya, 1972; Bhattacharya, Sowbhagya & Swamy, 1982). Varieties with high total and insoluble amylose contents (firm cooking rice) showed B type degradation; other high-amylose varieties gave A or B_1 type; low-amylose rice gave C type; intermediate-amylose rice showed mixed C type; and waxy rice gave a D-type disintegration. Thus, the alkali reaction type showed a good correlation with the amylose content and gave an approximate indication of rice quality.

In the 1950s, the GT was thought to be a fairly good index of rice quality (Halick & Kelly, 1959). It was later understood that the earlier correlation arose from incidental association. The GT was shown not to have any inherent relation either to the eating quality of rice, or its protein or amylose content (Bhattacharya, Sowbhagya & Swamy, 1982; Juliano, Bautista, Lugay & Reyes, 1964), although there have been some reports that the GT may play an important role in the quality of waxy rice (Juliano, Villareal, Perez, Villareal, Takeda & Hizukuri, 1987).

Researchers at IRRI developed a gel consistency test to differentiate rices with the same amylose contents but differences in eating quality. For this test, rice flour is dispersed in dilute alkali, and the gel is cooled and then allowed to flow in a tube. The length of the gel flow (long=soft gel, short=hard gel) had an inverse relationship with the amylose content (Cagampang, Perez & Juliano, 1973). It was believed by IRRI scientists that a combination of the amylose content and gel consistency tests would give a good indication of rice quality.

1.4.2 Instrumental measurements for predicting rice texture

With the development of mechanical techniques, including the mimicking our oral process, instrumental measurements were being used to predict food texture. One of the earliest important breakthroughs in food texture studies was from the work conducted by Szczesniak and her co-workers from the General Foods (now Kraft) in the 1960s. For the first time, a direct link between the mechanical properties of a food and its texture profile was established (Friedman, Whitney & Szczesniak, 1963). Using a so-called Texturometer, they

demonstrated that the force-displacement curve obtained from a double compression test (**Fig. 1.3**) gave a meaningful interpretation to a number of texture features: hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness. Szeczesniak's method was later named a Texture Profile Analysis.

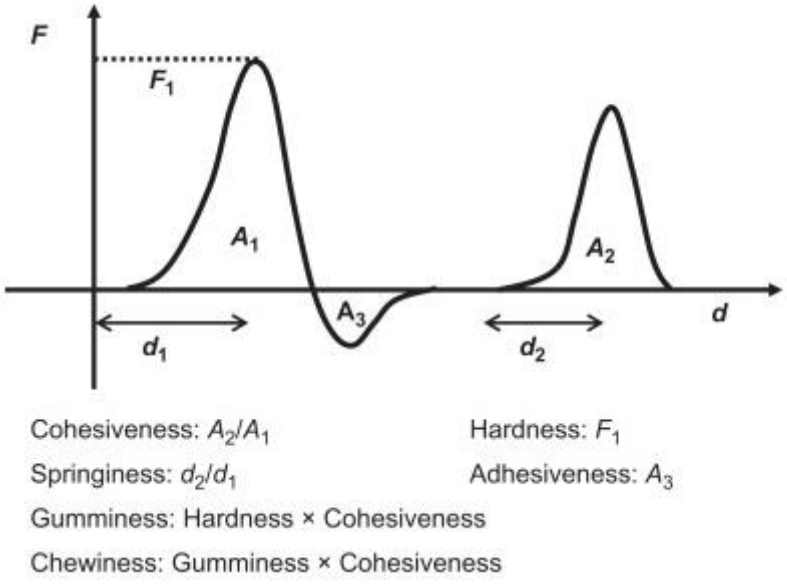

Figure 1.3 A typical force-displacement curve obtained from a double-compression test using the texture profile analysis approach. A single test is able to characterize a number of textural parameters (Chen, 2009).

For TPA analysis, difference cells can be employed. One of the most popular and reliable instrumental methods involves the use of an Ottawa extrusion cell (Juliano et al., 1984; Meullenet, Champagne, Bett, McClung & Kauffmann, 2000; Meullenet, Gross, Marks & Daniels, 1998). Extrusion tests are normally performed using various instruments, including the Ottawa texture measuring system (OTMS), a universal testing machine (Instron), a back extrusion cell and a texture analyser. With this empirical method, the maximum force during the extrusion process if recorded and generally correlates well with the sensory perception of hardness. However, the dimensions of the traditional Ottawa cell require rather large quantities of rice (~100 g) for evaluation. In many instances, rice breeders cultivate small experimental plots, and the small amounts of rice yielded is insufficient for such instrumental testing. As a result, compression tests, which require smaller sample sizes, performed between flat plates has been described by several researchers (Okabe, 1979). Normally, double compression for TPA uses a cylindrical plunger in conjunction with a texture analyser

(Lyon, Champagne, Vinyard & Windham, 2000), or single compression using a plunger in conjunction with a tensile testing machine (Juliano et al., 1981). While extrusion tests are commonly performed on bulk samples, compression tests are usually performed on a few kernels. Lyon et al. (2000) investigated sensory and instrumental relationships of the texture of cooked rice. In their experiments they used 1 g of cooked rice for compression tests; the correlations found between individual sensory descriptive attributes and instrumental texture profile parameters were weak. Sesmat & Meullenet (2001) predicted sensory texture characteristics of cooked rice using a compression test and a novel multivariate analysis method. They used 5 kernels in their compression test, and found 7 (cohesion of bolus, adhesion to lips, hardness, cohesiveness of mass, roughness of mass, toothpull, and toothpack) of the textural attributes were satisfactorily predicted with sensory results. Since compression tests are conducted on only a few kernels, tests performed on bulk samples yielded more consistent results (Juliano et al., 1981). Sitakalin & Meullenet (2000) also reported this problem when using extrusion and compression tests with spectral stress-strain analysis to predict the texture of cooked rice. The extrusion test provided more accurate predictions. However, compression tests present the main advantage of requiring smaller sample sizes than extrusion tests.

Another method used to assess functionality, including rice texture, is near-infrared spectroscopy (NIR). This analytical technique that has been used for the past 30 years to analyse various cereal grain constituents including moisture, protein, and oil (Williams, 1975). With regards to rice, NIR has been used to accurately predict the apparent amylose content (Villareal, De La Cruz & Juliano, 1994), the protein content (Delwiche, Mckenzie & Webb, 1996), and the surface lipids (Chen, Marks & Siebenmorgen, 1997). Because rice functionality depends on chemical constituents and their interaction, it seems logical that NIR could be used to directly assess functional characteristics such as paste viscosity. However, there has been less success at predicting functional attributes such as the alkali spreading value and viscosity (Delwiche, Mckenzie & Webb, 1996), or amylograms and cooking characteristics of short-grain Japanese rice (Natsuga & Kawamura, 2006). Windham et al. (1997) assessed the potential of NIR and NIR in combination with other physicochemical measurements for the determination of sensory texture attributes in whole-grain milled rice samples. NIR gave the best prediction results for the following texture attributes: manual adhesiveness, visual adhesiveness, and stickiness to lips, with a relative ability of prediction

of 0.57, 0.54, and 0.56, respectively. Additionally, the calibration of NIR plus physicochemical variables did not improve the predictability of sensory texture over NIR alone. Champagne et al. (2001) examined the ability of NIR to predict sensory texture attributes of diverse rice cultivars. Texture attributes (hardness, initial starchy coating, cohesiveness of mass, slickness, and stickiness) measured by panellists in the early evaluation phases were successfully predicted. They also concluded that the key wavelengths contributing to the models describing the texture attributes were wavelengths also contributing to models for amylose, protein, and lipid contents. Although NIR has been used to predict the quality of cooked rice texture with low to moderate success, it still works by predicting the compositions of amylose, protein, and lipids; however this is not a direct tool for measuring and predicting the texture of cooked rice.

1.4.3 Descriptive sensory evaluation

The texture profile method was first developed at the General Foods Research Center (Brandt, Skinner & Coleman, 1963; Szczesniak, Brandt & Friedman, 1963; Szczesniak & Kleyn, 1963). Brandt et al. (1963) defined a texture profile as "the sensory analysis of the texture complex of a food in terms of its mechanical, geometrical, fat and moisture characteristics, the degree of each present and the order in which they appear from first bite through complete mastication." Further interest in a detailed descriptive method developed as a result of the growth of new products and competition in the measurement and improved data processing systems. The quantitative descriptive analysis (QDA) method was developed, and represented an opportunity for sensory evaluation to satisfy these needs. This method included requiring the subjects to develop and agree on the language, the use of a scale to obtain measures of attribute strength, replication for assessing subject and attributes sensitivity, identifying specific product differences, and defined statistical analysis (Stone, Sidel, Oliver, Woolsey & Singleton, 1974; Stone & Sidel, 1998). Later, the spectrum descriptive analysis method was developed primarily from the Flavor Profile and Texture Profile methods; a description can be found in Meilgaard, Carr and Civille (2006). The training activities, as described, are quite extensive, reflecting the basic Flavor Profile and Texture Profile procedures, with particular reliance on the Texture Profile method of training subjects with specified standards of specified intensities. The training process is lengthy, requiring 6-8 hours per week for a period of 14 weeks, with 100 hours or more of training time per modality. This training time is described as necessary so as to enable the panel to be

universal, that is, to be able to evaluate all types of products (Meilgaard, Carr & Civille, 2006).

One of the first steps in developing universal methods is the adoption of sensory profile terminology by trained panellists. Mundo, Kosco, Juliano, Siscar and Perez (1989) reported the terminology granular, spongy, smooth-grain, sticky, watery, dry, soft bite, and firm-hard bite that was used by a European panel. Reported in the same study was terminology used by an American panel that included surface qualities before placing in the mouth like wetness, roughness, plumpness, and clumpiness, as well as firmness, rubberiness, crumbliness after five chews, and grainy and gritty particle characteristics. Terminology developed by a French panel included elasticity, stickiness, pastiness, mealiness, firmness, crunchiness, time in mouth, brittle texture and juiciness (Rousset, Pons & Pilandon, 1995). Later, a widely used terminology was developed in the late 1990s (Lyon, Champagne, Vinyard & Windham, 2000; Lyon et al., 1999; Meullenet, Gross, Marks & Daniels, 1998). The terms, including evaluation procedures and definitions of attributes, were clearly defined. The sensory texture profile included 16 sensory attributes that described rice texture at different phases of sensory evaluation, beginning with characteristics outside the mouth and ending with mouthfeel characteristics after rice was swallowed. Typical attribute definitions and techniques are listed in **Table 1.1**.

Table 1.1 Vocabulary for Sensory Texture Attributes of Cooked Rice (Champagne et al., 1999).

Phases/Attributes	Definition
PHASE I.	***Place 6-7 grains of rice in mouth behind front teeth. Press tongue over surface and evaluate.***
Initial Starchy Coating	amount of paste-like thickness perceived on the product before mixing with saliva (three passes).
Slickness	maximum ease of passing tongue over the rice surface when saliva starts to mix with sample.
Roughness	amount of irregularities in the surface of the product
Stickiness	degree to which the kernels adhere to each other
PHASE II.	***Place ½ teaspoon of rice in mouth. Evaluate before or at first bite.***
Springiness	degree grains return to original shape after partial compression
Cohesiveness	degree to which the grains deform rather than crumble, crack, or break when biting with molars.
Hardness	force required to bite through the sample with the molars.
PHASE III.	***Evaluate during chew.***
Cohesiveness of Mass	maximum degree to which the sample hold together in a mass while chewing.
Chewiness	amount of work to chew the sample.
Uniformity of Bite	evenness of force throughout bites to chew.
Moisture Absorption	amount of saliva absorbed by sample during chewing
PHASE IV.	***Evaluate after swallow.***
Residual Loose Particles	amount of loose particles in mouth.
Toothpack	amount of product adhering in/on the teeth.

1.4.4 New trends in oral processing, texture and mouthfeel:

Texture and mouthfeel play pivotal roles in product acceptability, and after the point at which the food enters the mouth (e.g., first bite of solids, initial thickness of liquids), it is currently difficult to predict these through measurements derived from imitative or empirical techniques such as TPA using a texture analyser or using fundamental rheological properties of food and beverages. On the other hand, food oral processing involves comminuting solid food to small particle sizes, mixing with saliva, and forming a bolus that is then swallowed and transferred to the stomach (Chen & Engelen, 2012). Regardless of the initial state of food, it undergoes a conversion to form a state that is rheologically suitable for swallowing in a highly sophisticated dynamic process (Van der Bilt, Engelen, Pereira, Van der Glas & Abbink, 2006). The organoleptic properties of food, including texture perception, depend on the constantly changing status of the food during oral processing as well as the changing

status of the salivary film coating oral surfaces and the saliva itself (Davies, Wantling & Stokes, 2009). Utilisation of knowledge of oral processing in the relevant *in vitro* measurement techniques is needed to provide mechanistic insights into texture/mouthfeel and can be used in food structure design; however these also require validation using *in vivo* studies and sensory science.

Food texture is regarded as a multidimensional sensory property that is influenced by the food's structure, rheology and surface properties (Kravchuk, Torley & Stokes, 2012). Food technologists have sought for a long time to instrumentally measure "texture", despite the caveat that it is multi-modal sensory percept. Historically, there are three key approaches: (i) imitative techniques (e.g., using so-called texture analysers), (ii) empirical methods that seek to align any sort of measurement to a sensory perception and (iii) fundamental mechanical properties of the food such as rheology and its underlying structure. Eating is a dynamic process, and studying the sequence of oral manipulations beyond the first bite has been very challenging. Combinations of *in vivo*, *ex vivo* (expectorating chewed food samples) and fully-imitative *in vitro* (i.e., mechanical chewers) measurements have been investigated (Foegeding et al., 2011; Foster, Grigor, Cheong, Yoo, Bronlund & Morgenstern, 2011). However, their use for rational food design is limited. In the assessment of the field, Stokes, Boehm and Baier (2013) concluded that *in vitro* strategies are required to specifically determine how various food components affect the dynamics of oral processing and ultimately texture perception. They take the approach that there are many deformation and transport processes occurring simultaneously during oral processing (van Vliet & Primo-Martin, 2011), and to uncover specific roles of ingredients these processes need to be broken down into specific fundamental steps. By breaking up oral processing in this manner, they consider it more likely to be able to isolate key *in vitro* measurements and methodologies that lead to greater insight into food structure design beyond the first bite. Brandt, Skinner and Coleman (1963) considered the different stages in which texture is perceived during oral processing: (i) Initial (first bite); (ii) masticatory (during chewing); and (iii) residual (texture during mastication). However, breaking it up into these three phases inhibits the development of *in vitro* techniques that seek to capture what happens to the food during oral processing. Stokes, Boehm and Baier (2013) suggest, for the purposes of developing *in vitro* approaches that enable rational design of solid foods, that oral processing is split into the following 6 stages: (i) first bite, (ii) comminution, (iii) granulation, (iv) bolus formation, (v) swallow and (vi)

residue. Recently, they depicted these stages, as given in **Fig. 1.4**. The changing status of food should be examined at each stage, and it should be noted that these processes overlap *in situ*, but studying them separately allows the underlying physics to be decoupled so that insights can be obtained on the specific functionality of food components.

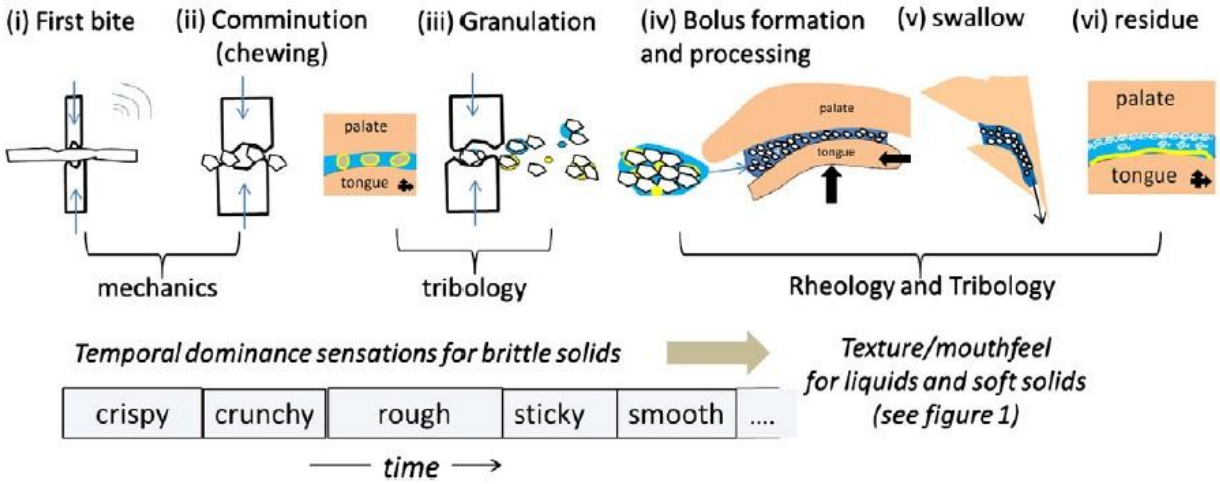

Figure 1.4 Depiction of 6 key stages proposed to occur during oral processing of solid food (Stokes, Boehm & Baier, 2013).

As is seen in **Fig. 1.4**, comminution (phase ii) is the crushing and grinding of the solid food into particulates. During comminution, food particles may rub oral surfaces leading to dry sensations, or liquid (aqueous or oil) may be released from the food that along with the saliva secreted from the oral cavity may act as a lubricant against irritation. Hence, there is a tribological interaction between the food particles and the oral surface, which is likely to play a major role in sensations such as grittiness and a rough mouthfeel. As solid foods are reduced to particulate form during chewing, they also aggregate via capillary bridging if small amounts of liquid are present. This is a process commonly referred to as granulation in powder processing (phase iii). As more saliva is secreted into the oral cavity, the particles become dispersed in saliva, i.e., a bolus forms; this may be considered a paste-like suspension (phase iv). At this stage, the particles can be potentially hydrated and subjected to enzymatic breakdown from amylase before the bolus is swallowed, and the bolus rheology will change with time as more saliva is continually secreted into the oral cavity and from the continual shear. The swallowing process (phase v) is thought to be controlled by a combination of particle size, moisture content and bolus rheology, all of which are critical to those with swallowing disorders. Following swallowing, left-over residue from the food can still contribute to mouthfeel/after feel perception along with the subsequent secretion of saliva

into the mouth, which is influenced by the food and beverages being consumed (phase vi) (Stokes, Boehm & Baier, 2013). In this way, one can see that there is a transformation from a rheology-dominated process (i.e., first bite) to a tribology-dominated process during oral processing, since surface interactions are of paramount importance. **Fig. 1.5** depicts the transition in film thickness of fluid-like foods or beverages between the oral surfaces as they are consumed, indicating that the process goes from a rheology-dominant deformation process to one where tribology (surface properties) dominates.

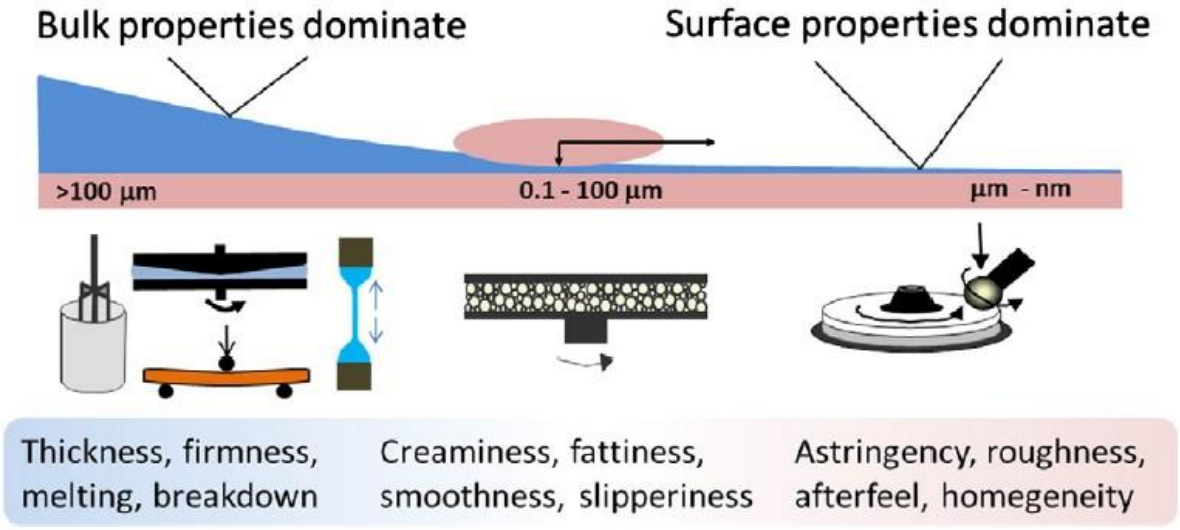

Figure 1.5 Depiction of the transition in film thickness of fluid-like foods (food bolus) or beverages between oral surfaces as they are consumed (Stokes, Boehm & Baier, 2013).

These new trends and techniques in the understanding of oral processing, texture and mouthfeel also give new insights for the *in vitro* assessment of rice texture. Since current *in vitro* methods for assessing and predicting cooked rice texture, like TPA, are still limited to mimicking the first bite, and the rice oral processing involves comminuting solid food to small particle sizes, mixing with saliva, and forming a bolus that is then swallowed and transferred to the stomach, rheology and tribology techniques may supply some new information to explore the dynamic process of rice oral processing.

1.5 Thesis proposal on the understanding of texture of cooked rice

Until now, hardness and stickiness are the most important attributes for evaluating the texture of cooked rice. Some regulations of hardness and stickiness were confirmed, for example, high-amylose rice always has a harder and less sticky texture while low-amylose rice always has a sticky and soft texture. However, this is not applicable in the case that rices with similar

amylose content show different textural properties, or the case that rice can also show the same texture while their amylose content are totally different. At this point in time, the mechanisms of structural understanding of hardness and stickiness have been ambiguous. One possible reason for this lack of conclusiveness is the difficulties of characterizing starch structure, especially the fine structure of amylose.

Human oral processing involves comminuting solid food to small particle sizes, mixing with saliva, and forming a bolus that is then swallowed and transferred to the stomach. Currently, TPA is still the most commonly used instrumental method to measure the texture of cooked rice, but only hardness and stickiness are valuable attributes from TPA measurements which just mimic the first bite of rice kernels. The poor repeatability of this method is also reported when conducted on freshly cooked rice, due to the rapid retrogradation of rice starch with rice decreasing temperature, which, consequently, resulted in more replicates and complex sample preparation needed to obtain statistically meaningful data. Furthermore, the range of geometries available for texture analysers has also meant that standard fixtures and procedures are not always used, which makes it difficult to compare studies. Therefore, an improved instrumental method for evaluating cooked rice is needed.

Therefore, the overall objectives of this thesis is to explore the molecular mechanistic reasons for the hardness and stickiness of cooked rice grains, increase understanding of the human textural perception of cooked rice, and develop an improved instrumental method to evaluate and/or predict the texture of cooked rice. From the literature review above, three questions are proposed as follows to provide the framework for fulfilling this goal:

Question 1: what is the structural basis for the hardness for cooked rice?

Hardness is the most important textural attribute of cooked rice. It is still ambiguous on the molecular- (structural-) level explanation of the mechanism of hardness of cooked rice.

Amylose content has previously used as the determinant of hardness of cooked rice. Now, it is true that for rice samples with a wide range of amylose content, higher amylose content correlates with harder texture of the cooked rice. However, when rice samples with similar amylose content have different hardness values, or when rice samples containing different amylose content have similar hardness, the correlation between amylose content and harder texture of cooked rice is not applicable. Further, as discussed in **Section 1.3.1**, it has been

proposed that the long amylopectin branches are more important in determining the hardness of cooked rice. Therefore, the structural basis of hardness of cooked rice is still unclear.

Question 2: What is structural basis for the stickiness of cooked rice?

Stickiness is another important attribute of cooked rice. This attribute is less commonly studied than is hardness. Stickiness has been reported to be related to the amylopectin content and/or structure, which is why stickiness always negatively correlated with hardness: for example, high-amylose rices have less amylopectin, which causes a harder and less sticky texture, whereas waxy rices contain no amylose and show the stickiest texture. Hence hardness and stickiness are always like the two sides of a coin. However, rices with similar amylopectin (or amylose) contents can still show different stickiness.

Further, the reported stickiness of a rice sample depends on which part of rice is measured or perceived. For example, the stickiness measured by human lips, or the stickiness measured by TPA, reflect the sticky attribute on the surface of rice kernels, and would probably be most related to the amylopectin structure on the surface of the rice kernel (some amylopectin would have leached out of the rice kernel during heating). However, the stickiness perceived during chewing reflects the stickiness of the whole rice kernel, and would be more related to the amylopectin structure of the whole rice grain.

Thus, for the stickiness measured by the usual instrumental method (TPA), it is important to explore starch leaching behaviour and the corresponding fine structure, especially for the rice samples with similar amylopectin (amylose) content but different sticky attributes.

Question 3: How to overcome disadvantages of current TPA method and develop an improved instrumental method for the evaluation of the texture of cooked rice?

TPA is the most commonly used instrumental method to measure the texture of cooked rice and other food products. This method has been employed with some success and, in some cases, provides data that relate closely to sensory evaluation data. However, its limitations restrain further applications. The texture analyser is used to obtain the force-displacement curve by a double-compression test of typically two rice kernels, which is less reliable and accurate than a test performed on bulk samples. The poor repeatability of this method is also reported when conducted on freshly cooked rice, due to the rapid retrogradation of rice starch with rice decreasing temperature, which, consequently, resulted in more replicates and complex sample preparation needed to obtain statistically meaningful data. Furthermore, the

range of geometries available for texture analysers has also meant that standard fixtures and procedures are not always used, which makes it difficult to compare studies. An improved instrumental method is thus needed for evaluating more sensory attributes of cooked rice.

Therefore, to answer these three questions, the whole project will be implemented in three separate but linked sub-projects:

Chapter 2--- The importance of amylose and amylopectin fine structure for textural properties of cooked rice grains;

Chapter 3--- The molecular structural features controlling stickiness in cooked rice, a major palatability determinant;

Chapter 4--- Instrumental measurement of cooked rice texture by dynamic rheological testing and its relation to the fine structure of rice starch;

The second chapter will help us understand which part of starch structure is responsible for hardness of cooked rice, the third chapter will explain the causal reasons for the stickiness between cooked rice grains using understanding of the rice swelling process, starch leaching and leached starch structures, the forth chapter will supply an improved instrumental method for evaluating and/or predicting the texture of cooked rice; this method will also be applied to look for mechanistically meaningful correlations between starch structure and textural properties.

Chapter 2 The importance of amylose and amylopectin fine structure for textural properties of cooked rice grains

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Chapter abstract: Statistically and causally meaningful relationships are established between starch molecular structure (the molecular distribution of branched starch and the chain length distribution of debranched starch) and texture (hardness and stickiness) of cooked rice grains. The amounts of amylose chains with degree of polymerization (DP) 100-20000, and of long amylopectin chains, positively correlated with hardness, while amylopectin chains with DP<70 and amylose molecular size both showed negative correlations with hardness (p<0.05). There was also a significant negative correlation between stickiness and the amounts of long amylopectin chains (p<0.01). For rices with similar amylose content, the amount of amylose chains with DP 1000-2000 positively correlated with hardness while size negatively correlated with hardness (p<0.05). This indicates for the first time that, regardless of amylose content, rice varieties with smaller amylose molecular sizes and with higher proportions of long amylose chains have a harder texture after cooking.

2.1 Introduction

Rice is a major staple food world-wide. In recent years, consumer preferences have shifted towards better-quality rice, particularly towards varieties with good eating quality. Each country, and often region, prefers rice with a particular suite of quality traits (Calingacion et al., 2014). The textural attributes of cooked milled rice are of prime importance to its eating quality. Texture is a multi-parameter sensory property, with hardness and stickiness as the most commonly determined parameters for cooked rice (Patindol, Gu & Wang, 2010). In addition to sensory evaluation by human panels, textural properties of cooked rice are most commonly measured by instruments such as a textural analyser (Cameron & Wang, 2005; Champagne et al., 1998).

Cooked rice texture is affected by a wide range of factors, such as the amylose content (Juliano, Onate & Del Mundo, 1972), postharvest processing (Champagne et al., 1998), and cooking method (Leelayuthsoontorn & Thipayarat, 2006). Among these, starch structure has an important role in rice texture (Cameron & Wang, 2005; Ramesh, Zakiuddin Ali & Bhattacharya, 1999). Starch is a branched glucose polymer comprising two types of molecules: amylopectin and amylose. Amylopectin molecules are highly branched with a vast number of short branches and relatively large molecular weights, ~$10^{7–8}$, whereas amylose has a smaller molecular weight (~$10^{5–6}$) with a few long branches (Gilbert, Witt & Hasjim, 2013). The amylose content has been considered to be the most important determinant of the eating quality of rice since the mid-1980s (Bhattacharya & Juliano, 1985). In the mid-1990s, it was proposed that the texture of cooked rice is also related to the fine structure of amylopectin (Ramesh, Zakiuddin Ali & Bhattacharya, 1999). Ong and Blanshard (1995) determined the amylose content and the amylopectin fine structure of 11 cultivars of non-waxy rices, and confirmed that the texture of cooked rice was critically controlled by the proportion of the longest and shortest amylopectin chains but not the intermediate ones. Ramesh et al. (1999) analysed the starch structure of 7 rice varieties, concluding that the content of all long linear chains, including amylose if any, governed the texture of cooked rice.

The present study is an in-depth consideration of the mechanisms of starch structural effects on rice texture. A novel factor in the present paper is an examination of the role of the fine structure of amylose(Gilbert, Witt & Hasjim, 2013), which is a significant factor in starch digestibility(Syahariza, Sar, Hasjim, Tizzotti & Gilbert, 2013).

There are several techniques for starch fine structural analysis: fluorophore-assisted carbohydrate electrophoresis (FACE), high-performance anionic-exchange chromatography (HPAEC), and size-exclusion chromatography (SEC - sometimes termed gel-permeation chromatography or GPC) (Wu, Witt & Gilbert, 2013). FACE is the optimal method for determining the chain-length distributions (CLDs) of amylopectin. SEC suffers from the problems of band-broadening, calibration, and inaccuracies in the Mark-Houwink relation used to related molecular size to degree of polymerization (DP), which are all obviated with FACE. However, because of the inability to quantitatively detect chains above a relatively low DP, currently ~ 180 , FACE and HPAEC can only give information on amylopectin chains and (for FACE) the shortest amylose chains. SEC does not suffer from the same restriction and can therefore be used for the measurement of amylose fine structure (Gilbert, Witt & Hasjim, 2013).

The objective of this study is to obtain a mechanistic understanding of the relationship between starch (amylopectin and amylose) fine structure and textural properties (hardness and stickiness) of cooked rice grains. Since the starch granular and crystalline structures are greatly disrupted by the cooking process, only the grain composition and starch molecular structure will be analysed here. The structural features are the CLDs of the individual polymeric chains of debranched amylose and amylopectin, and the molecular size distributions of whole (fully branched) starch. The rice varieties chosen for the present study have a wide range of amylose content. Among these, 7 rice varieties were deliberately chosen to contain similar amylose content but which differ in sensory properties, in order to discover any correlations that are separate from those due to amylose content alone. The hardness and stickiness of the cooked rice were determined from texture profile analysis using a texture analyser. The results will aid understanding of the role of starch fine structure in determining the textural properties of cooked rice grains.

2.2 Materials and methods

2.2.1 Materials

Twelve milled rice grain samples were chosen from a collection of rice varieties with known phenotypes and genotypes for quality traits (**Table 2.1**). Protease from *Streptomyces griseus* (type XIV), and LiBr (ReagentPlus) were purchased from Sigma-Aldrich Pty. Ltd. (Castle Hill, NSW, Australia). Isoamylase (from *Pseudomonas sp.*) and a D-glucose (glucose

oxidase/peroxidase; GODOP) assay kit were purchased from Megazyme International, Ltd. (Wicklow, Ireland). A series of pullulan standards with peak molecular weights ranging from 342 to 2.35 $\times 10^6$ were from Polymer Standards Service (PSS) GmbH (Mainz, Germany). Dimethyl sulfoxide (DMSO, GR grade for analysis) was from Merck Co. Inc. (Kilsyth, VIC, Australia). All other chemicals were reagent-grade and used as received.

2.2.2 Cryogenic grinding of rice grains

Rice grains were ground into flour with a cryogenic mill (Freezer/Mill 6850; SPEX, Metuchen, NJ) in a liquid nitrogen bath as the cryogenic medium, following the procedure described by Syahariza et al. (2013) to minimize the degradation to starch granules (Tran, Shelat, Tang, Li, Gilbert & Hasjim, 2011).

2.2.3 Composition of rice grains

The starch content of the rice grains was analysed from the ground rice flour using a GOPOD assay kit. The crude lipid content was determined by Soxhlet extraction, following AOAC method 920.39C (AOAC, 2002). The crude protein content of the rice grains was calculated from the nitrogen content of the rice flour, obtained using a LECO CNS2000 auto analyser (LECO Corporation, St. Joseph, MI) with a conversation factor of 5.95 (Jones, 1941).

2.2.4 Starch extraction from rice grains

All starch samples were extracted and dissolved in a DMSO solution with 0.5% (w/w) LiBr (DMSO/LiBr) at a concentration of 2 mg/mL, following a method described elsewhere (Syahariza, Li & Hasjim, 2010; Tran, Shelat, Tang, Li, Gilbert & Hasjim, 2011). A protease and sodium bisulfite solution was used first, followed by a centrifugation step, to remove protein from the rice flour. The treated rice flour was agitated in DMSO/LiBr and the starch then precipitated from the resulting soluble portion by adding 10 mL of ethanol; samples were then centrifuged at 4000 g for 10 min. This is better than extracting starch from rice grains using an alkaline solution, which can act as a catalyst for starch hydrolysis, especially when heating and mixing are involved (Chiou, Martin & Fitzgerald, 2002; Wu, Li & Gilbert, 2014). The extracted starch in the DMSO/LiBr solution was stored at room temperature for subsequent analysis by SEC and debranching for CLD analysis.

2.2.5 Molecular size distribution of whole branched starch molecules

The structure of extracted whole starch molecules was characterized using an Agilent 1100 Series SEC system (Agilent Technologies, Waldbronn, Germany) equipped with GRAM 30 and 3000 analytical columns (PSS) and a refractive index (RI) detector (RID-10A, Shimadzu Corp., Kyoto, Japan) following a method described elsewhere (Cave, Seabrook, Gidley & Gilbert, 2009; Liu, Halley & Gilbert, 2010). The molecular size distribution of branched starch was plotted as the weight distribution, $w_{br}(\log R_h)$, against the hydrodynamic volume Vh (the separation parameter for SEC), or the equivalent hydrodynamic radius, R_h; $V_h = 4/3\ \pi R_h^3$. For branched starch molecules, as for any branched polymer, there is no unique relation between size and the molecular weight. The assumption of universal calibration for SEC is that the elution time of the analyte depends only on its V_h and not on its structure, whence one has for two polymers, a sample and a standard, the relation:

$$K_{\text{standard}} M^{\alpha(\text{standard})+1} = K_{\text{sample}} M^{\alpha(\text{sample})+1} \quad (1)$$

Pullulan standards with known peak molecular weights were used for calibration to obtain a relationship between SEC elution volume and V_h of starch molecules following the Mark-Houwink equation:

$$V_h = \frac{2}{5} \frac{K M^{1+\alpha}}{N_A} \quad (2)$$

Here N_A is Avogadro's constant and M is the molecular weight. The Mark-Houwink parameters K and α of pullulan in DMSO/LiBr solution at 80 ℃ are 2.424×10^{-4} dL g^{-1} and 0.68, respectively (Cave, Seabrook, Gidley & Gilbert, 2009).

2.2.6 Starch debranching and measuring the CLD of debranched starch using SEC

The extracted starch (~4 mg) was dissolved in 0.9 mL of deionized water and then mixed with 2.5 μL isoamylase (1000 U/mL), 0.1 mL acetate buffer solution (0.1 M, pH 3.5), and 5 μL sodium azide solution (0.04 g mL^{-1}). The mixture was incubated at 37 ℃ for 3 h. The debranched starch suspension was then heated in a water bath at 80 ℃ for 2 h after being neutralized with 0.1 M NaOH solution, and then freeze-dried overnight. The dried debranched starch was dissolved in DMSO/LiBr solution for SEC analysis.

To obtain SEC distributions of debranched starch, GRAM 100 and GRAM 1000 columns (PSS) were used, with the same pullulan standards and procedure used to calibrate the SEC for whole branched molecules. The SEC weight distribution, $w(\log X)$, obtained from the DRI signal was plotted against X (DP), with X being determined using the Mark-Houwink

relationship (see Equation 1), with M = 162.2(X–1)+18.0 (162.2 is the molecular weight of the anhydroglucose monomeric unit and 18.0 is that of the additional water in the end groups); the Mark-Houwink parameters K and α for linear starch chains in the eluent of DMSO/LiBr at 80 ℃ are 1.5 × 10^{-4} dL g^{-1} and 0.743, respectively. For a linear polymer (such as debranched starch), the number distribution (obtained by debranching), $N(X)$, is related to the corresponding weight distribution by (Castro, Dumas, Chiou, Fitzgerald & Gilbert, 2005):

$$w(\log X) = X^2 N_{\mathrm{de}}(X) \quad (3)$$

The degree of branching (DB) is obtained from the CLD using the relation DB = 1/(number average of $N_{\mathrm{de}}(X)$).

2.2.7 Fitting amylopectin number CLD with a biosynthesis model

The number distribution was fitted using the Wu-Gilbert model (Wu & Gilbert, 2010), which considers the CLD from a biosynthetic perspective. In this model, the number distribution is assumed to be controlled solely by the action of three types of starch biosynthesis enzyme: starch synthase (SS), starch branching enzyme (SBE), and starch debranching enzyme (DBE). The kinetic equations of the rates of action of each enzyme determine the number distribution of branches, that is, $N_{\mathrm{de}}(X)$, giving the relative number of chains of the debranched starched comprising X monomer units. There are several different sets of the three types of enzymes, denoted "enzyme sets": for example, there are four isoforms of branching enzyme, SBEI, SBEII, SBEIIa and SBEIIb, and a particular enzyme set contains only one of these four (plus one each of the various types of starch synthase and debranching enzymes). The overall $N(X)$ is the sum of the contributions of each enzyme set. By fitting the number CLD of amylopectin with this model, a series of parameters can be obtained characterizing the enzymatic processes of the amylopectin biosynthesis. In addition, SBE can only form branches with lengths longer than a certain minimum DP, X_{min}, and the length of moiety retained after branching must be more than a certain minimum DP, X_0. The activity ratios of SBE/SS and DBE/SS are denoted β and γ, respectively. From the mathematical development, for given values of X_0 and X_{min}, each value of γ is associated with a value of β, so that γ is eliminated from the fitting (Wu & Gilbert, 2010). For SEC CLD data (where some features of the fine structure are masked by band broadening), the CLD of amylopectin branches with DP ≤ 100 can be fitted by three enzyme sets, denoted enzyme sets 1, 2 and 3, and the relative contributions of enzyme set 2 and 3 to enzyme set 1 are termed $h_{2/1}$ and $h_{3/1}$, respectively

(Witt, Doutch, Gilbert & Gilbert, 2012). The role of phosphorylase in forming enzyme complexes between different enzymes and isoforms of these (Tetlow et al., 2008; Tetlow et al., 2004) is acknowledged as contributing to the action of each enzyme set. Fitting is implemented with publicly-available code (Wu & Gilbert, 2013).

2.2.8 Amylose content

The amylose content of rice starch was determined from the SEC weight distributions of debranched starch. This was taken as the ratio of the area under the curve (AUC) of amylose branches (defined to have DPs $\geq$ 100) to the AUC of the entire distribution (including both amylopectin and amylose branches). This method has been shown to be more accurate than the iodine colorimetric method (Fitzgerald et al., 2009; Vilaplana, Hasjim & Gilbert, 2012).

2.2.9 Preparation of cooked rice

Rice (100 g, 14% moisture content) was rinsed with distilled water three times. Distilled water was then added to the rice to give a rice-to-water weight ratio of 1:1.6. The cooking process was conducted using the pre-set cooking setting of a rice cooker (Kambrook Rice Express, VIC, Australia), followed by a 10 min holding period at the warming setting. The top 1 cm layer of cooked rice and rice adhering to the sides of rice cooker were not used. Cooked rice for sampling was taken directly from the middle of each cooker, transferred to a pre-warmed (120 ℃) glass bowl, and mixed thoroughly while minimizing kernel breakage. The cooked rice was then cooled to room temperature (~25 ℃) for textural measurements.

2.2.10 Texture profile analysis (TPA)

A 1 g subsample of cooked rice grains was weighed and placed as a single layer of grains on the base plate. A two-cycle, force-versus-distance compression program was used to measure and calculate using a TA.XT-Plus Texture analyser with a 35 mm cylindrical probe attachment (Stable Micro Systems Ltd., Surrey, UK). The probe was allowed to descend at 1 mm/s, return, and then repeat the compression cycle. Compression was set to 80% strain. For each cooking replicate, texture measurements were conducted six times. Parameters recorded from the test curves were hardness (force at the peak of the first curve) and stickiness (area of the negative force curve).

2.2.11 Statistical analysis

For each structural measurement, duplicated analyses were performed for each sample. All data were reported as mean ± standard deviation (SD) using analysis of variance (ANOVA) with Tukey's pairwise comparisons. Significant differences of the mean values were determined at $p < 0.05$. The textural measurements were analysed in duplicate for each sample. One-way analysis of variance (ANOVA) and Pearson as well as Spearman rank correlation methods were carried out using SPSS V. 16.0 software (SPSS Inc., Chicago, IL). The means of duplicated measurements were used for the correlation analysis.

2.3 Results and discussion

2.3.1 Rice composition

Rice compositions are presented in **Table 2.1**. The total starch content ranges from 78% to 86%, the protein content from 6.5% to 9.4%, and total lipid content is between 0.2% and 0.9%. Between these different rice samples, there are some significant differences in the total starch, protein, and lipid content. The starch, protein, and lipid content of rice samples in this study are within the ranges previously reported for rice.

Table 2.1 Chemical composition of rice samples*

Varieties	Abbreviation code	Sample collection	Country of origin	Total starch (%)	Total protein (%)	Total lipid (%)
Hom Mali Niaow	HMN	Lab collection	Australia	81.1 ±0.4^{a,b}	8.4 ±0.1^{f}	0.3 ±0.0^{a-c}
Tailand Jasmine	TJ	Supermaket	Thailand	81.1 ±1.4^{a,b}	6.9 ±0.0^{b}	0.9 ±0.2^{f}
Kangaroo	KG	Lab collection	Australia	81.2 ±1.3^{a,b}	7.3 ±0.0^{c,d}	0.7 ±0.0^{d,e}
Phka Rum Duol	PRD	Lab collection	Australia	78.0 ±1.1^{a}	9.4 ±0.0^{g}	0.2 ±0.0^{a}
Kyeema	KM	Lab collection	Australia	81.1 ±1.4^{a,b}	8.2 ±0.0^{e}	0.8 ±0.0^{e,f}
LanGI	LG	Lab collection	Australia	80.7 ±1.0^{a,b}	8.2 ±0.0^{e}	0.5 ±0.0^{b-d}
Sunrice Medium Grain	SMG	Supermaket	Australia	82.9 ±0.2^{b,c}	7.0 ±0.0^{b}	0.3 ±0.1^{a,b}
Golden way	GW	Lab collection	Australia	85.7 ±0.5^{c}	7.2 ±0.0^{b,c}	0.6 ±0.0^{d,e}
Viet 8	V8	Lab collection	Australia	79.0 ±1.1^{a,b}	7.4 ±0.1^{d}	0. 5 ±0.1^{b-d}
Basmati	BM	Supermaket	India	79.0 ±1.2^{a,b}	8.3 ±0.1^{e,f}	0.3 ±0.1^{a-c}
Sunrice Long grain	SLG	Supermaket	Thailand	86.1 ±1.3^{c}	6.5 ±0.1^{a}	0.5 ±0.1^{c,d}
Swarna	SN	Lab collection	India	79.7 ±0.9^{a,b}	8.6 ±0.0^{f}	0.6 ±0.1^{d,e}

*Mean ±SD is calculated from duplicates. Values with different letters in the same column are significantly different with $p < 0.05$.

2.3.2 Starch molecular structure

Typical SEC weight distributions, $w_{br}(\log R_h)$, of whole branched starch from all rice grain samples are shown in **Fig. 2.1**, normalized to the peak maximum of amylopectin; the fully branched distribution of all rice samples display two populations of α-glucans: amylose (R_h up to ~100 nm) and amylopectin (R_h between 100 and 4000 nm) (**Fig.** 2.**1**). There is another small peak/shoulder peak at R_h ~3 nm, which may be residual proteins (Syahariza, Li & Hasjim, 2010). These residual proteins possibly arise from incomplete hydrolysis by protease during the starch extraction procedure and are not relevant to this study, and this component of $w_{br}(\log R_h)$ is not considered further. The amylose component of the whole molecule distributions is expressed as the value of R_h at the amylose peak maximum and the average R_h (between 0 and 100 nm) of amylose, $\overline{R_h}$, as defined elsewhere (Vilaplana & Gilbert, 2010), while the corresponding for the amylopectin component is expressed as the value of R_h at the amylopectin peak maximum. As presented in **Table 2.2**, there are statistically significant differences in both the R_h at the amylose peak maximum and $\overline{R_h}$ of amylose among different rice varieties, whereas there is little significant difference in the R_h at the amylopectin peak maximum between samples. As shown in **Fig. 2.1**, Hom Mali Niaow (HMN) is a waxy rice with the lowest amylose content, and thus has the lowest AUC in the amylose region (even lower than that of the residual protein); however this starch, while its amylose content is very small, has the largest molecular size in the R_h at the amylose peak maximum and $\overline{R_h}$ of amylose (**Table 2.2**). Given that genetically this variety cannot produce amylose (Wanchana, Toojinda, Tragoonrung & Vanavichit, 2003), it is possible that the polymers found in the region where amylose molecules are found are possibly small molecules of amylopectin that co-elute in the amylose region of the chromatogram. In contrast, high-amylose rice starches such as SLG and SN, which have amylose peaks close to or even higher than the amylopectin peaks, have relatively low amylose molecular sizes with a smaller R_h at the amylose peak maximum and a smaller $\overline{R_h}$ across the amylose region (**Fig. 2.1**). It has been pointed out (Fitzgerald et al., 2009; Vilaplana, Hasjim & Gilbert, 2012) that amylose content cannot be accurately measured from the whole-molecule size distribution because of co-elution of the molecules, but is best measured by the AUC from the debranched distribution as above.

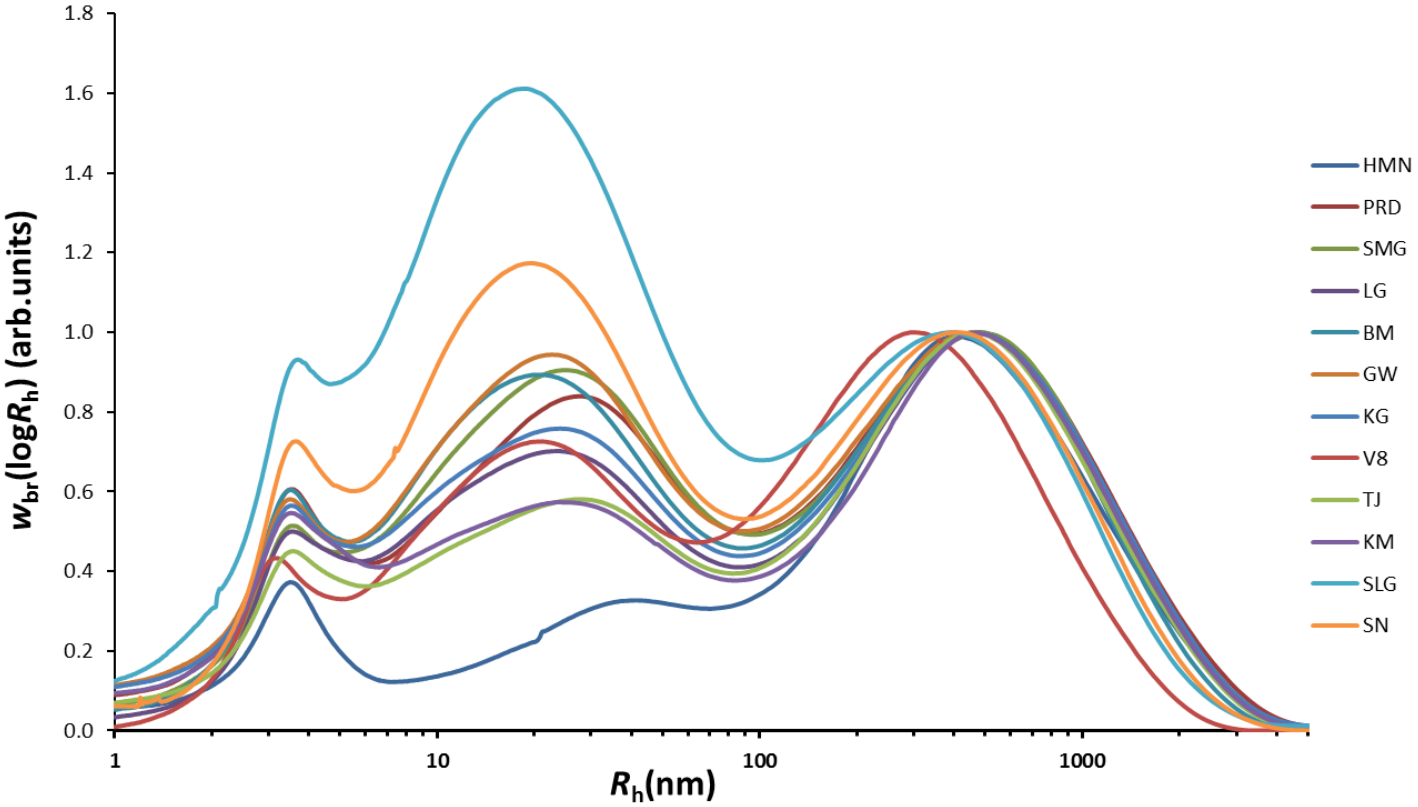


Figure 2.1 SEC weight distributions of whole starch, $w_{br}(\log R_h)$, extracted from all rice grain samples, and normalized to the amylopectin peak.

Table 2.2 Starch molecular parameters extracted from SEC and model fitting parameters for all rice samples.*

Rice varieties	Branched starch parameters					Debranched starch parameters				
	DB	R_h/nm at Am peak maximum	Average R_h/nm for Am	R_h/nm at Ap peak maximum	$h_{Am/Ap}$	DP of peak maximum		DP of peak maximum	Height of peak maximum	
						X_{AP1}	X_{AP2}	X_{Am}	h_{Ap2}/h_{Ap1}	h_{Am}/h_{Ap1}
HMN	4.92 ± 0.01 [a]	41.2 ± 2.2 [f]	24.7 ± 0.9 [e]	403.5 ± 16.1 [a,b]	0.33 ± 0.00 [a]	20.3 ± 0.2 [b]	43.0 ± 0.3 [b,c]	-	0.64 ± 0.01 [c,d]	-
TJ	4.43 ± 0.10 [a]	27.6 ± 1.5 [e]	19.8 ± 0.7 [b-d]	454.6 ± 43.5 [b]	0.58 ± 0.01 [b]	20.6 ± 0.1 [b]	43.3 ± 0.3 [c]	738.0 ± 63.8 [a,b]	0.67 ± 0.00 [d]	0.06 ± 0.00 [a]
KG	4.34 ± 0.00 [a]	24.0 ± 0.2 [c-e]	18.5 ± 0.1 [a-c]	458.1 ± 37.6 [b]	0.76 ± 0.02 [b-d]	20.4 ± 0.2 [b]	42.4 ± 0.0 [a-c]	918.6 ± 111.5 [a,b]	0.61 ± 0.00 [b,c]	0.08 ± 0.00 [b,c]
PRD	4.62 ± 0.06 [a]	27.8 ± 0.8 [e]	21.4 ± 0.5 [d]	468.8 ± 11.1 [b]	0.84 ± 0.03 [c,d]	19.5 ± 0.5 [a]	40.9 ± 0.2 [a]	2595.8 ± 0.0 [e]	0.58 ± 0.00 [a,b]	0.08 ± 0.00 [b,c]
KM	4.61 ± 0.33 [a]	24.6 ± 1.6 [c-e]	20.0 ± 0.4 [c,d]	477.3 ± 33.8 [b]	0.57 ± 0.01 [b]	20.5 ± 0.1 [b]	42.4 ± 0.0 [a-c]	1290.1 ± 117.9 [b-d]	0.57 ± 0.02 [a,b]	0.07 ± 0.01 [a,b]
LG	4.89 ± 0.07 [a]	23.8 ± 0.2 [b-e]	18.9 ± 0.7 [b,c]	460.9 ± 0.0 [b]	0.72 ± 0.09 [b,c]	20.2 ± 0.3 [a,b]	41.9 ± 0.6 [a-c]	2254.7 ± 0.0 [d,e]	0.55 ± 0.00 [a]	0.09 ± 0.00 [c-e]
SMG	4.63 ± 0.17 [a]	25.1 ± 0.7 [d,e]	18.8 ± 0.3 [b,c]	488.7 ± 5.8 [b]	0.91 ± 0.03 [c,d]	20.2 ± 0.2 [a,b]	41.7 ± 0.3 [a-c]	2053.0 ± 124.0 [d,e]	0.60 ± 0.02 [b,c]	0.09 ± 0.01 [d-f]
GW	4.86 ± 0.12 [a]	22.8 ± 1.3 [a-d]	18.3 ± 0.4 [a-c]	468.7 ± 0.0 [b]	0.94 ± 0.03 [d]	20.2 ± 0.0 [a,b]	41.7 ± 1.2 [a-c]	1973.9 ± 135.8 [c-e]	0.60 ± 0.01 [b,c]	0.10 ± 0.01 [e,f]
V8	4.69 ± 0.41 [a]	20.8 ± 0.7 [a-d]	16.5 ± 0.1 [a]	296.2 ± 3.3 [a]	0.73 ± 0.06 [b,c]	20.2 ± 0.2 [a,b]	41.3 ± 0.3 [a,b]	1984.3 ± 118.8 [c-e]	0.59 ± 0.01 [b]	0.10 ± 0.00 [f,g]
BM	4.65 ± 0.11 [a]	20.4 ± 0.9 [a-c]	17.9 ± 0.8 [a,b]	465.0 ± 16.4 [b]	0.89 ± 0.08 [c,d]	20.3 ± 0.2 [b]	41.7 ± 0.6 [a-c]	1021.4 ± 74.3 [b,c]	0.57 ± 0.00 [a,b]	0.11 ± 0.00 [g]
SLG	4.57 ± 0.30 [a]	18.8 ± 0.7 [a]	16.6 ± 0.1 [a]	388.4 ± 22.2 [a,b]	1.61 ± 0.0.08 [f]	20.5 ± 0.1 [b]	42.1 ± 0.0 [a-c]	662.2 ± 43.3 [a,b]	0.66 ± 0.01 [d]	0.18 ± 0.00 [i]
SN	4.50 ± 0.00 [a]	19.6 ± 0.0 [a,b]	18.0 ± 0.0 [a,b]	410.8 ± 9.5 [b]	1.18 ± 0.01 [e]	20.3 ± 0.2 [b]	42.4 ± 0.3 [a-c]	764.1 ± 74.0 [a,b]	0.60 ± 0.01 [b,c]	0.15 ± 0.00 [h]

Debranched starch parameters				Model fitting parameters				
Amylose content	DP (100-20000)							
	100<*X*<1000	1000<*X*<2000	2000<*X*<20000	$h_{2/1}$	$h_{3/1}$	$\beta_{(i)}$	$\beta_{(ii)}$	$\beta_{(iii)}$
1.40 ± 0.01 [a]	0.85 ± 0.30 [a]	0.02 ± 0.01 [a]	0.04 ± 0.01 [a]	0.0474 ± 0.0042 [b-d]	0.0017 ± 0.0001 [a]	0.0761 ± 0.0060 [a]	0.0626 ± 0.0023 [b]	0.0433 ± 0.0004 [c]
13.34 ± 0.01 [b]	5.02 ± 0.54 [b]	0.96 ± 0.05 [b]	1.20 ± 0.05 [b]	0.0513 ± 0.0005 [c,d]	0.0023 ± 0.0001 [a,b]	0.0752 ± 0.0015 [a]	0.0616 ± 0.0021 [b]	0.0395 ± 0.0023 [b,c]
18.21 ± 0.00 [c]	6.25 ± 0.02 [b,c]	1.42 ± 0.05 [c]	1.99 ± 0.09 [c]	0.0468 ± 0.0000 [b-d]	0.0023 ± 0.0001 [a,b]	0.0820 ± 0.0033 [a]	0.0578 ± 0.0020 [a,b]	0.0368 ± 0.0029 [a-c]
18.38 ± 0.00 [c,d]	5.82 ± 0.25 [b,c]	1.40 ± 0.02 [c]	2.38 ± 0.09 [c-f]	0.0396 ± 0.0004 [a]	0.0020 ± 0.0002 [a,b]	0.082 ± 0.0001 [a]	0.0595 ± 0.0005 [a,b]	0.0358 ± 0.0028 [a-c]
18.99 ± 0.02 [c,d]	6.76 ± 1.09 [b,c]	1.32 ± 0.04 [c]	2.03 ± 0.25 [c,d]	0.0503 ± 0.0025 [c,d]	0.0028 ± 0.0006 [b,c]	0.0783 ± 0.0051 [a]	0.0596 ± 0.0017 [a,b]	0.0347 ± 0.0022 [a,b]
20.18 ± 0.00 [c,d]	6.46 ± 0.24 [b,c]	1.67 ± 0.00 [d]	2.48 ± 0.04 [d-f]	0.0479 ± 0.0002 [b-d]	0.0022 ± 0.0001 [a,b]	0.0835 ± 0.0043 [a]	0.0622 ± 0.0016 [b]	0.0366 ± 0.0029 [a-c]
20.94 ± 0.01 [c,d]	6.71 ± 0.37 [b,c]	1.70 ± 0.09 [d]	2.61 ± 0.04 [e,f]	0.0450 ± 0.0012 [a-c]	0.0021 ± 0.0002 [a,b]	0.0823 ± 0.0013 [a]	0.0604 ± 0.0000 [b]	0.0370 ± 0.0033 [a-c]
21.78 ± 0.01 [c-e]	7.34 ± 0.58 [c,d]	1.76 ± 0.06 [d]	2.60 ± 0.04 [e,f]	0.0455 ± 0.0005 [a-d]	0.0025 ± 0.0001 [a-c]	0.0790 ± 0.0021 [a]	0.0605 ± 0.0003 [b]	0.0362 ± 0.0001 [a-c]
21.88 ± 0.01 [d,e]	7.31 ± 0.42 [c,d]	1.84 ± 0.01 [d]	2.59 ± 0.01 [e,f]	0.0439 ± 0.0007 [a,b]	0.0025 ± 0.0001 [a-c]	0.0745 ± 0.0031 [a]	0.0580 ± 0.0002 [a,b]	0.0377 ± 0.0006 [a-c]
24.95 ± 0.00 [e]	9.03 ± 0.44 [d]	2.09 ± 0.00 [e]	2.75 ± 0.17 [f]	0.0456 ± 0.0019 [a-d]	0.0032 ± 0.0001 [c]	0.0802 ± 0.0006 [a]	0.0575 ± 0.0001 [a,b]	0.0330 ± 0.0010 [a,b]
29.94 ± 0.00 [f]	13.52 ± 0.38 [e]	2.43 ± 0.05 [f]	2.23 ± 0.08 [c-e]	0.0490 ± 0.0002 [b-d]	0.0048 ± 0.0001 [d]	0.0795 ± 0.0061 [a]	0.0546 ± 0.0021 [a]	0.0308 ± 0.0007 [a]
29.45 ± 0.01 [f]	12.53 ± 0.20 [e]	2.41 ± 0.03 [f]	2.59 ± 0.19 [e,f]	0.0518 ± 0.0009 [d]	0.0050 ± 0.0000 [d]	0.0801 ± 0.0001 [a]	0.0547 ± 0.0008 [a]	0.0318 ± 0.0002 [a,b]

Mean ± SD is calculated from duplicate measurements. Values with different letters in the same column are significantly different with $p < 0.05$.

Typical SEC weight distributions of debranched starch, $w_{de}(\log R_h)$, from all grain samples are presented in **Fig. 2.2A**. The same information is presented in **Fig.** 2.**2B** as the CLD, in terms of the number distribution N_{de} (X); those different representations of the same data bring out different features of the distribution. All weight and number distributions are normalized to the highest amylopectin branch peak. The components with $X < 100$ are defined as amylopectin branches, while those with $X \geq 100$ are defined amylose chains (Vilaplana, Hasjim & Gilbert, 2012). The SEC weight distributions of debranched starch from all rice grain samples show the usual features. There are two large peaks of amylopectin branches and one smaller peak of amylose branches. The first peak (denoted Ap1) is the global maximum, which comprises the shorter amylopectin braches with lengths up to a DP of 30 (R_h ~0.5–2 nm); these are confined to one amorphous/crystalline lamella. The second peak or shoulder (denoted Ap2) are longer amylopectin branches with DPs ranging from 30 to 99 (R_h ~2–4 nm), which span more than one crystalline lamella. The amylose CLDs have DPs ranging from 100 to 20000 and an R_h ranging from 4 to 300 nm. As seen elsewhere (Wang, Hasjim, Wu, Henry & Gilbert, 2014; Ward, Gao, de Bruyn, Gilbert & Fitzgerald, 2006), there are significant differences in these amylose peaks between different rice varieties. These differences are probably due to differences in potentially discrete enzymatic processes in plant starch biosynthesis.

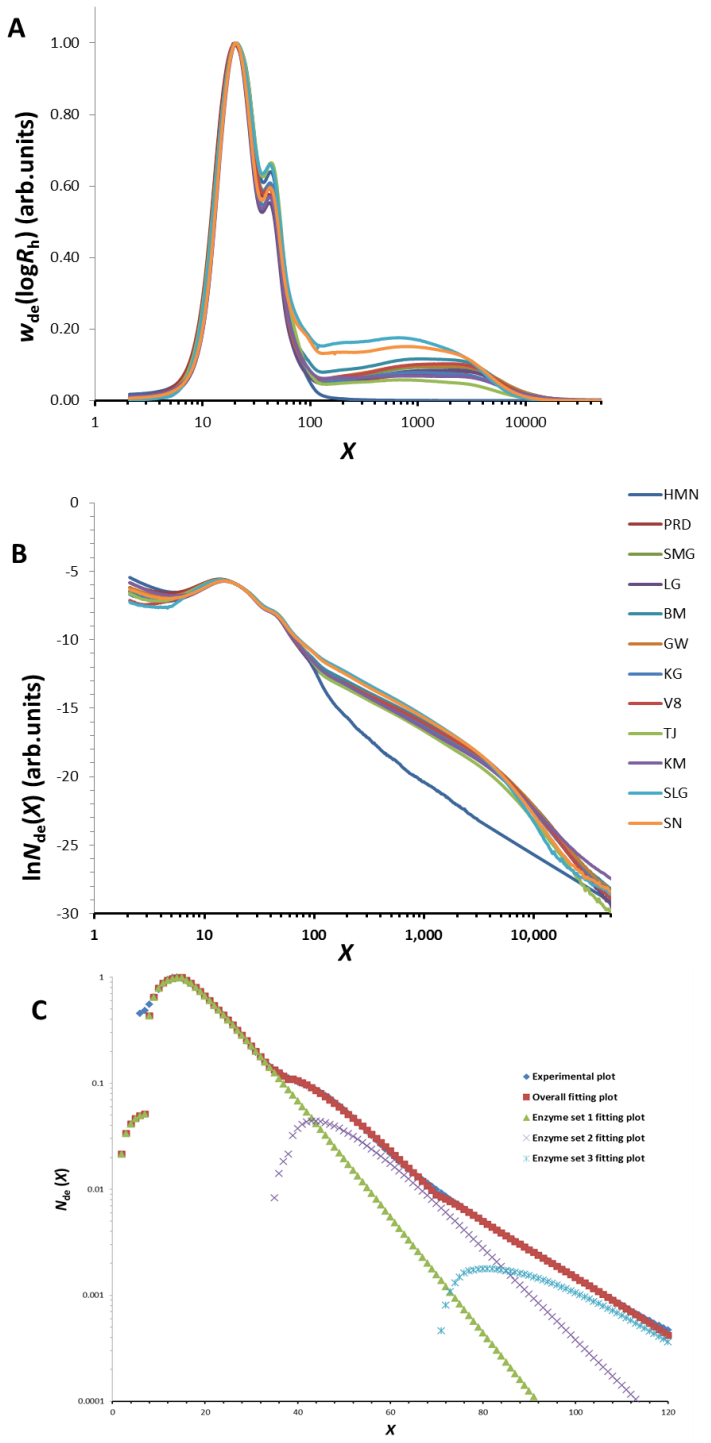


Figure 2.2 (A) SEC weight and (B) number CLDs of debranched rice starches. All distributions are normalized to the highest amylopectin peak. (C) Plot of the experimental results from SEC (in blue) and the model fitting (in red) of number CLD of amylopectin branches for sample HMN. The total CLD, $N_{de}(X)$, is the sum of the components from enzyme sets 1 and 2 (green and purple, respectively); note that the plot has a logarithmic scale.

To compare the fine structure of the various starches, in addition to fitting with the Wu-Gilbert model (which only is applicable to amylopectin), a set of empirical parameters was used, that had been defined previously(Syahariza, Sar, Hasjim, Tizzotti & Gilbert, 2013). These are the DP at the maximum of each peak, donated X_{Ap1}, X_{Ap2}, and X_{Am}, and the height ratio of each maximum relative to that of Ap1, $h_{Ap2/Ap1}$ and $h_{Am/Ap1}$. The DP at the maximum of each peak reflects the relative size of chains in each group of branches, while the height ratio of each peak maximum relative to Ap1 represents the relative amount of chains in each group of branches. Because of SEC band broadening (Gilbert, Witt & Hasjim, 2013), the two peaks/shoulders from the shorter and longer amylopectin branches and the two peaks from the longer amylopectin branches and the shorter amylose branches overlap. To gain more information on differences in the amylose fine structure between samples and its responsible properties, the X range of amylose is further subdivided into 3 different fractions, $100 \leq X < 1000$, $1000 \leq X < 2000$, and $2000 \leq X < 20000$. The percentage of the AUC for each fraction was also calculated (Vilaplana, Hasjim & Gilbert, 2012).

Amylose is synthesized through the *Waxy* (*Wx*) gene, which encodes granule bound starch synthase. Different haplotypes of the *Wx* gene are defined by single nucleotide polymorphisms (SNPs) at exon 1 and 6, which affect the amount of amylose accumulated (Chen, Bergman, Pinson & Fjellstrom, 2008). Waxy varieties contain a duplication in exon 2 of the *Wx* that completely disables transcription, so waxy varieties produce no amylose (Wanchana, Toojinda, Tragoonrung & Vanavichit, 2003). The varieties used in the present paper have previously been genotyped at the *Wx* locus (Calingacion et al., 2014). As shown in **Table 2.2,** all rice varieties containing amylose can be divided into 3 categories which agree with the *Wx* haplotype, defined by functional SNPs at exons 1 and 6 of the *Wx*: low-amylose rice which all contain T at exon 1 (TJ, PRD, SMG, LG, GW, KG, V8, and KM amylose content~0-19%); one variety, BM, with haplotype G-C of the *Wx* gene with intermediate amylose (amylose content~20-25%); and high amylose rice, with *Wx* haplotype G-A (SLG, and SN, amylose content >25%). There are significant structural differences between these 3 categories of rice. Compared to X_{Ap1} and X_{Ap2}, X_{Am}, which measures the DP at the peak maximum, varies much more significantly (**Table 2.2**). For rice varieties with intermediate and high amylose content, and with G at exon 1 of the *Wx* gene, X_{Am} tends to be smaller than for those with low amylose and T at exon 1 of the *Wx*. This could indicate that rice with a functional allele of *Wx* contains more short branches. It would be interesting to

explore whether this is a characteristic of all high amylose rices, which could provide insight into functional differences between the *Wx* haplotypes.

The amylopectin number CLDs (**Fig. 2.2B**) were fitted with the amylopectin biosynthesis model , with all the features reproduced well in the fitted number CLDs for all rice samples (see Figure S4.1 of the Supporting Information). The model provides information on the activities of the core starch synthesizing enzymes and gives insights into starch biosynthesis (Wang, Hasjim, Wu, Henry & Gilbert, 2014). As shown in **Fig. 2.2C**, the group of amylopectin chains of X<34, which are confined to one crystalline lamella (single-lamella), was dominated by enzyme set 1, while enzyme set 2 dominated DPs in the range between 34 and 70, which are trans-lamellar branches that span one crystalline lamella and the adjacent amorphous lamella. Correspondingly, enzyme set 3 was largely responsible for synthesizing the branches from DP 70 to 100 . From the model fitting, three β values ($\beta_{(i)}$, $\beta_{(ii)}$ and $\beta_{(iii)}$), each representing the relative activity of SBE to SS within each enzyme set, and another set of parameters $h_{2/1}$ and $h_{3/1}$ reflecting the relative contributions of enzyme sets 2 and 3 to that of enzyme set 1 were obtained. As shown in the "model fitting" section of **Table 2.2**, the $\beta_{(i)}$ values of rice starches between different rice varieties were not significantly different, while $\beta_{(ii)}$, $\beta_{(iii)}$, $h_{2/1}$, and $h_{3/1}$ differed significantly. This indicated that the effects of enzyme sets 2 and 3 on the number CLDs are more significant than those of enzyme set 1, suggesting that the differences in the proportion of longer amylopectin branches between all starch samples, as observed from the SEC weight CLDs (**Fig. 2.2A**), are mainly due to the differences in the reaction rates of enzyme sets 2 and 3. As indicated from **Table 2.2**, high and intermediate amylose rices tend to have higher values of $h_{2/1}$ and $h_{3/1}$, and smaller values of $\beta_{(ii)}$ and $\beta_{(iii)}$, suggesting that enzyme sets 2 and 3 have a lower SBE activity, and/or a higher SS activity, consequently causing a higher proportion of long amylopectin branches. These three varieties are known to carry haplotype 1 of SSIIa, (G/G/GC), which is a more active form of the enzyme (Cuevas et al., 2010), therefore suggesting that SS activity explains the values. This method of obtaining statistically useful information by fitting to the biosynthesis-based model is very much to be preferred over the older method of dividing the CLD into arbitrarily chosen DP ranges and using the proportions of each; this older method is empirical, and different results can be obtained if different ranges are chosen.

2.3.3 Textural properties of cooked rice grains

During cooking, rice granules absorb water and swell to much more than their original size. This granule expansion causes ruptures in the grain, leading to a decrease in the hardness. Furthermore there is well-documented evidence that amylose and amylopectin molecules leach into the surrounding water above the gelatinization temperature (Cuevas, Gilbert & Fitzgerald, 2010). These leached amylose and amylopectin molecules are likely to contribute to the stickiness of cooked rice (Leelayuthsoontorn & Thipayarat, 2006).

In this study, all rice varieties are cooked in the same rice/water ratio to avoid the effect of water content on the textural properties of cooked rice, as it has been shown that greater amounts of water will decrease the rice's hardness (Bett-Garber, Champagne, Ingram & McClung, 2007). As shown in **Fig. 2.3A** and **2.3B**, cooked rice grains from different rice varieties exhibit significant differences in their hardness and stickiness. It is noteworthy that, for these rice varieties, hardness is negatively correlated with stickiness (**Fig. 2.3C**). Juliano et al. (1981) measured the texture of 10 milled cooked rices using instrumental methods from 11 laboratories. They also found that hardness showed significant negative correlation with stickiness, showing that hardness was positively correlated with amylose content, whereas stickiness was negatively correlated with amylose content (Juliano et al., 1981). This is consistent with other reports (Cameron & Wang, 2005; Patindol, Gu & Wang, 2010).

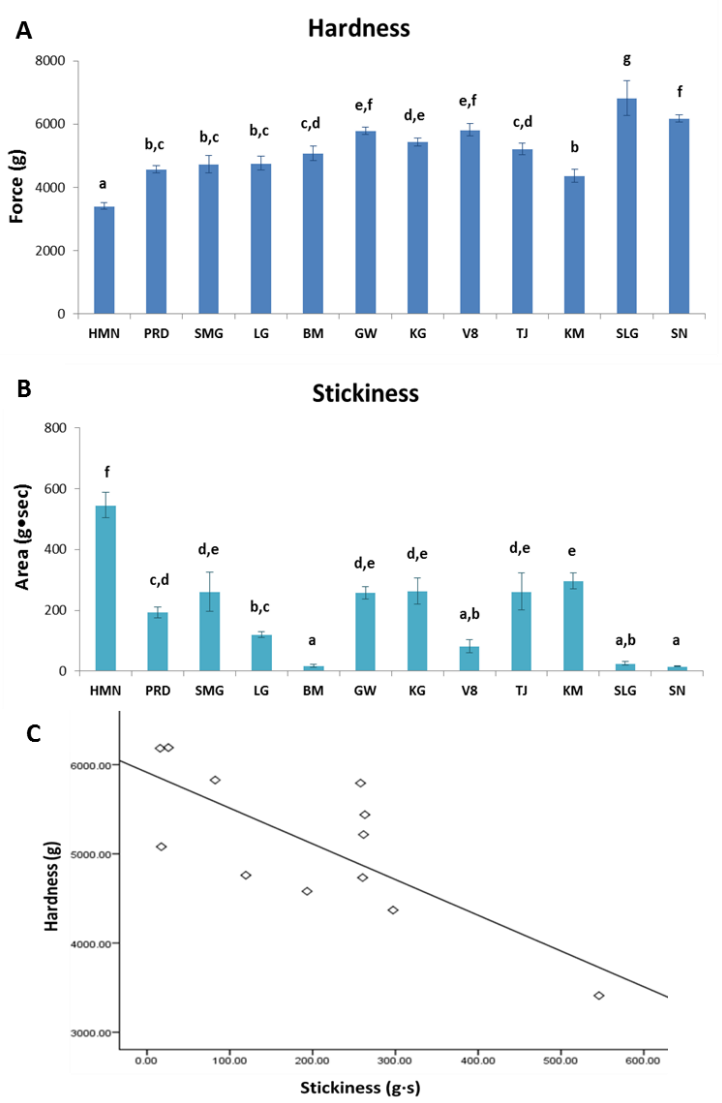


Figure 2.3 A) Hardness of all rice varieties; B) Stickiness of all rice varieties; C) Scatter plot between hardness and stickiness with a significant coefficient of -0.753 (p<0.05). Different letters above the column represent the significant difference with p< 0.05.

2.3.4 Structure - texture relations

The coefficients from Pearson's and Spearman's rank correlation tests between the textural properties (hardness and stickiness) and the starch structural parameters of all samples are summarized in **Table 2.3**. Pearson's correlation test reflects linear correlations, while Spearman's rank correlation test is able to detect non-linear correlations. The correlations of rice samples with similar amylose content (rice category with low amylose (PRD, SMG, LG, GW, KG, V8, and KM)) are also presented in **Table 2.3** to demonstrate statistically significant differences in the correlations when a narrow range of amylose contents was used.

The influence of starch fine structural features on the texture of cooked rice was also investigated. This is the first such examination of these effects, especially in regards to the fine structure of amylose. Among these starch structural parameters, eleven independent structural variables were used to describe the fine molecular structure of whole and debranched starch. These were: R_h at the amylose peak maximum; the $\overline{R_h}$ of the amylose component; the height ratio of amylose to amylopectin peak, $h_{Am/Ap}$, in the SEC weight distributions of whole starch; three branch-chain lengths (X_{Ap1}, X_{Ap2}, and X_{Am}); two height ratios ($h_{Ap2/Ap1}$ and $h_{Am/Ap1}$) of the peak maxima of debranched starch; and the proportions of chains in the three subdivided sections of the CLDs ($100 \leq X < 1000$, $1000 \leq X < 2000$, and $2000 \leq X < 20000$). Five model fitting parameters were used to describe the structure of amylopectin branches from the insights of starch biosynthesis: three enzymatic activity ratios of SBE/SS ($\beta_{(i)}$, $\beta_{(ii)}$ and $\beta_{(iii)}$), and two relative contributions of enzyme sets 2 and 3 to enzyme set 1 ($h_{2/1}$ and $h_{3/1}$); details of the fitting are given in **Fig. S2.1** of the Supplementary Data.

Table 2.3 Correlation coefficients between textural properties (hardness and stickiness) and the structural attributes.

Structural attributes	All rice samples				Rice samples with similar amylose content			
	Pearson		Spearman		Pearson		Spearman	
	Hardness	Stickiness	Hardness	Stickiness	Hardness	Stickiness	Hardness	Stickiness
Grain composition								
Starch (%)	0.295	0.109	0.212	0.353	0.307	0.541	0.179	0.536
Protein (%)	-0.473	0.059	-0.427	-0.112	-0.605	-0.184	-0.5	-0.143
Lipid	0.316	0.052	0.364	0.231	0.185	0.384	0.107	0.571
Fine starch molecular structures								
Am content	0.811**	-0.905**	0.692*	-0.860**	0.568	-0.368	0.571	-0.536
X_{AP1}	0.184	0.044	0.12	0.212	0.159	0.292	-0.216	0.541
X_{AP2}	-0.209	0.462	0.007	0.375	-0.126	0.546	-0.144	0.667
X_{Am}	-0.47	0.241	-0.609*	0.118	-0.154	-0.528	-0.214	-0.643
h_{Ap2}/h_{Ap1}	0.15	0.269	0.259	0.343	0.614	0.343	0.393	0.321
h_{Am}/h_{Ap1}	0.718*	-0.800**	0.664*	-0.873**	0.708	-0.506	0.75	-0.714
$h_{2/1}$	0.188	-0.025	0.182	0.007	-0.146	0.334	-0.25	0.536
$h_{3/1}$	0.695*	-0.689*	0.692*	-0.629*	0.224	0.208	0.25	0.214
$\beta_{(i)}$	-0.001	-0.256	-0.168	-0.217	-0.513	0.241	-0.321	-0.071
$\beta_{(ii)}$	-0.738**	0.720**	-0.636*	0.650*	-0.416	-0.059	-0.143	-0.143
$\beta_{(iii)}$	-0.654*	0.821**	-0.378	0.629*	0.629	-0.614	0.679	-0.429
$100<X<1000$	0.817**	-0.857**	0.685*	-0.748**	0.615	-0.107	0.464	-0.071
$1000<X<2000$	0.820**	-0.922**	0.776**	-0.874**	0.641*	-0.556	0.857*	-0.607
$2000<X<20000$	0.605*	-0.785**	0.259	-0.636*	0.32	-0.493	0.321	-0.393
Whole starch molecular structures								
DB	-0.427	0.279	-0.371	0.028	0.065	-0.416	0.429	-0.714
R_h/nm at Am peak maximum	-0.843**	0.892**	-0.818**	0.804**	-0.761*	0.391	-0.857*	0.464
Average Am R_h/nm	-0.878**	0.827**	-0.853**	0.734**	-0.807*	0.408	-0.929**	0.286
$h_{Am/Ap}$	0.756**	-0.721**	0.678*	-0.692*	0.355	0.107	0.286	-0.036

* Correlations are significant at $p < 0.05$; ** Correlations are significant at $p < 0.01$.

Among these structural parameters, $h_{Am/Ap1}$, $100 \leq X < 1000$, $1000 \leq X < 2000$, $2000 \leq X < 20000$ and $h_{Am/Ap}$ are all directly related to the amylose content. For the correlation of all rice samples, all of these parameters, along with the amylose content, show similar and significant positive correlations with hardness and negative correlations with stickiness. This is consistent with past conclusions that found that the amylose content is the most important determinant of rice textural quality (Juliano, Onate & Del Mundo, 1972). Additionally, both Pearson and Spearman correlation tests show that the parameters of $100 \leq X < 1000$ and $1000 \leq X < 2000$ have higher correlation coefficients, especially for $1000 \leq X < 2000$. This indicates that rices with higher amylose contents, especially higher proportions of amylose branches ranging from 1000 to 2000 DP, yield harder texture after cooking. Correspondingly, the parameters of $\beta_{(i)}$, $\beta_{(ii)}$, $\beta_{(iii)}$, $h_{2/1}$, and $h_{3/1}$ represent the content of amylopectin chains. Both $\beta_{(ii)}$ and $\beta_{(iii)}$ significantly and positively correlated with stickiness while $h_{3/1}$ showed strong and negative correlation with stickiness, indicating that rices with more amylopectin short chains and less amylopectin trans-lamella chains tend to be more sticky. As expected, $h_{3/1}$, reflecting the proportion of long trans-lamella chains with DP $70 \leq X < 100$, shows a significant and positive correlation with hardness, which is also consistent with other reports (Ong & Blanshard, 1995a). On the other hand, R_h at the amylose peak maximum and $\overline{R_h}$ of the amylose region are both parameters reflecting the molecular sizes of whole amylose molecules. As summarized in **Table 2.3**, the amylose molecular size is significantly and negatively correlated with hardness and positively correlated with stickiness. Because the amylose content correlates so strongly with the texture and structure of cooked rice, many of the observed correlations may simply be due to amylose content. Therefore in order to find correlations that are independent of the amylose content, 7 varieties with similar amylose contents were selected from all of the varieties and statistically re-analysed using Pearson and Spearman correlation tests.

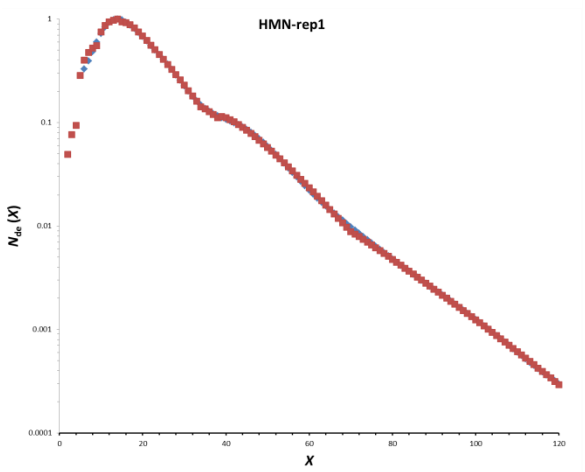
For rice samples with similar amylose contents, as expected there was no significant correlation between the texture of the cooked rice and the amylose content (**Table 2.3**). However, the whole amylose molecular size parameters (R_h at amylose peak maximum and amylose $\overline{R_h}$) and the proportions of amylose branches ranging between 1000 and 2000 DP still correlated significantly with hardness (**Table 2.3**). This indicates that, independent of the amylose content, rice varieties with higher proportions of amylose branches ranging from 1000 to 2000 and with smaller whole amylose molecules are harder. Furthermore, although

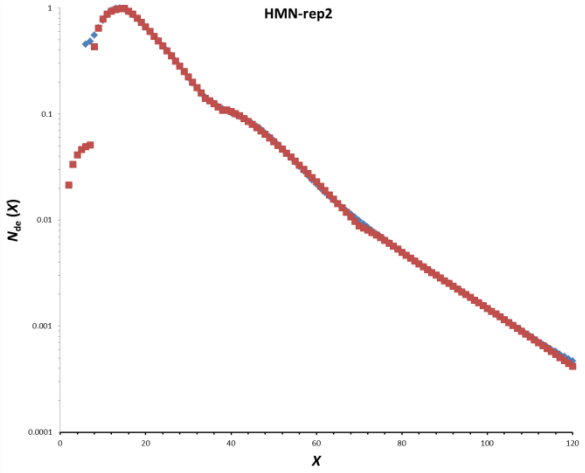
the stickiness of these rice samples with similar amylose content was significantly different (**Fig. 2.3B**), there was no significant correlation between stickiness and any of the structural parameters (**Table 2.3**). These new understandings of the fine structure of amylose content pave the way for a much deeper understanding of the important properties of rice, such as gel consistency, they offer new and significant phenotypes for understanding the eating quality of rice, and they could enable scientists to unravel the genetic and biochemical pathways that lead to high quality rice.

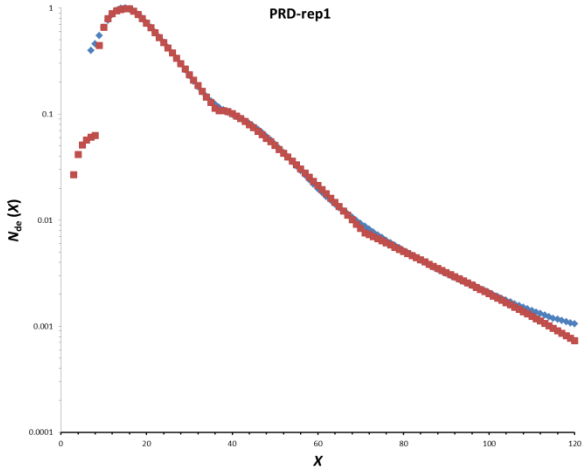
2.4 Conclusions

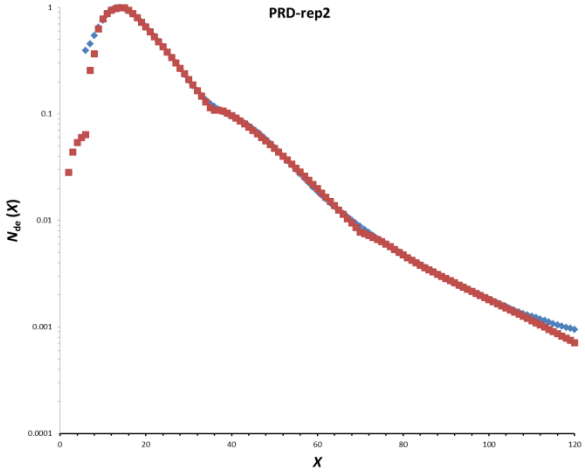
This study gives a new perspective on the relationship between the fine structure of amylose and amylopectin and the texture of cooked rice. The correlations found here support past studies that have found the amylose content to be important for the texture of cooked rice. Our study also shows, for the first time, that the whole amylose molecular size and the proportion of amylose branches ranging from 1000 to 2000 DP have significant effects on the hardness of cooked rice. A smaller amylose molecular size and a higher proportion of amylose branches with DP from 1000 to 2000 were found in the varieties with intermediate and high amylose, and these also led to an increase in hardness. How these structural features affect amylose leaching during cooking, and/or the degree of starch granule swelling during heating, may help explain the mechanism for this increase in hardness. Additionally, the amylopectin content and short chains of amylopectin are significantly and positively correlated with the stickiness of cooked rice samples with a wide range of amylose content. This study provides valuable information for further research to progress our understanding of (i) the relationship between the fine structure of starch and the sensory properties of rice, and (ii) the genetic regulation of the starch biosynthetic pathway.

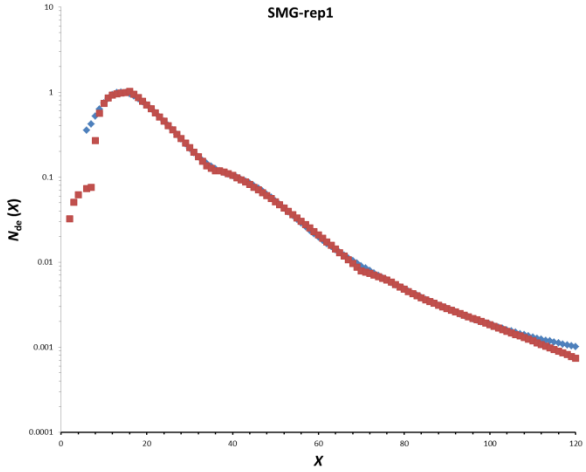
2.5 Supplementary data

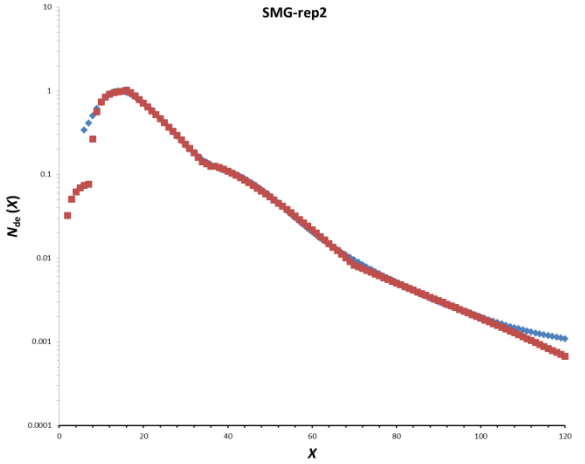


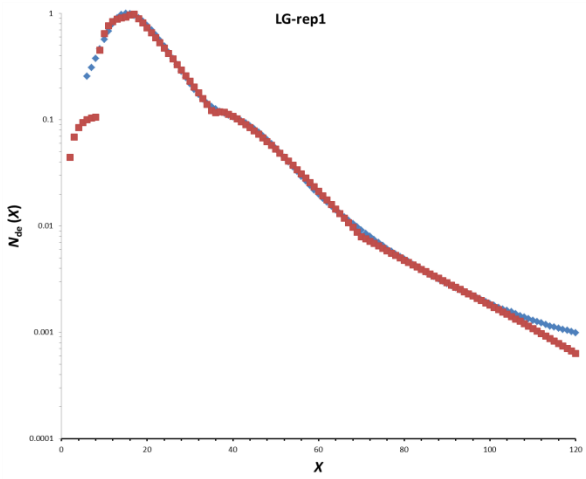








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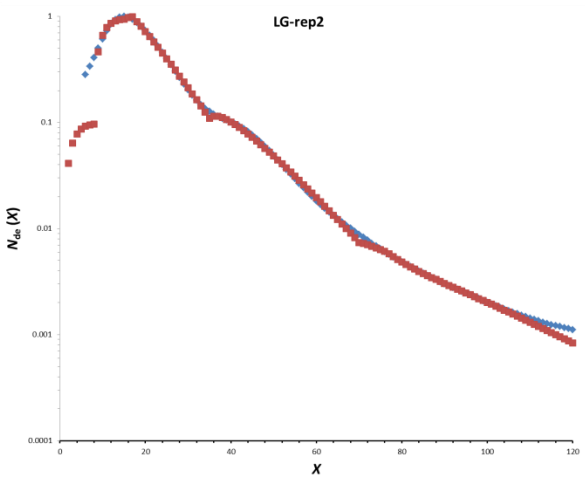
X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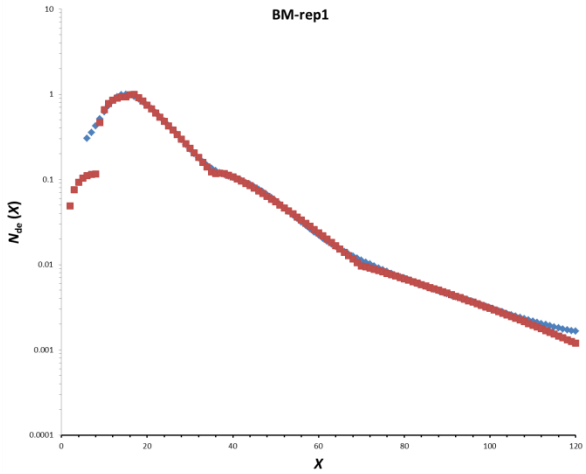
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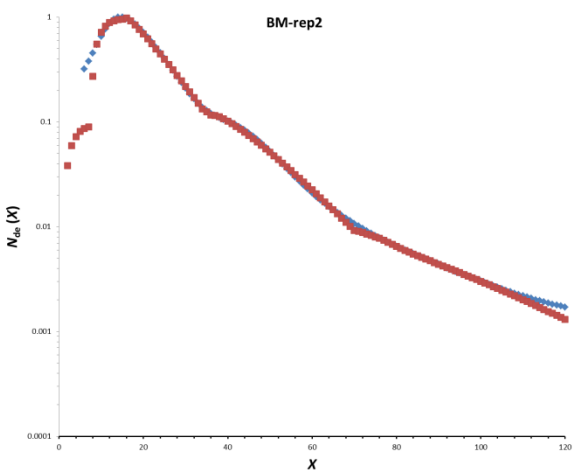
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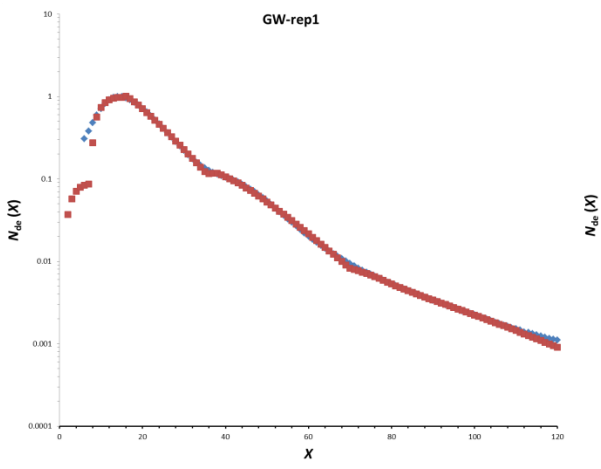
X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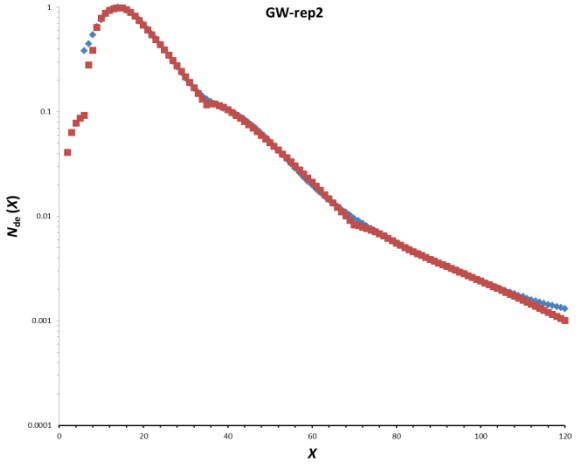
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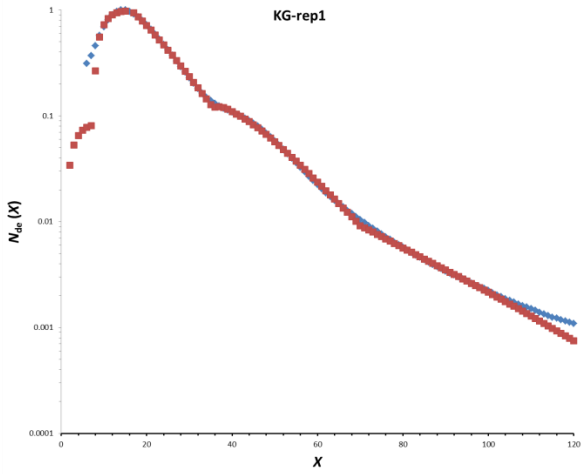
X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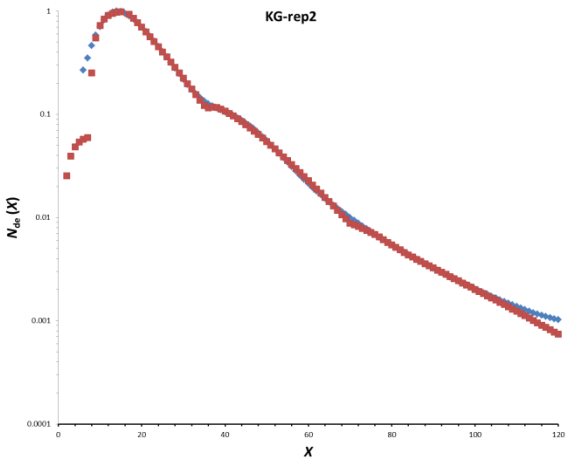
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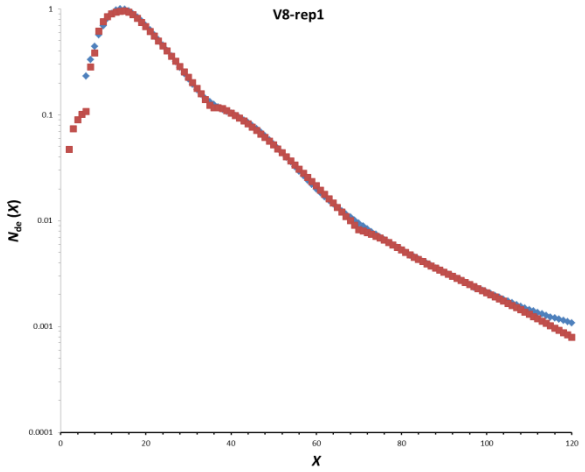
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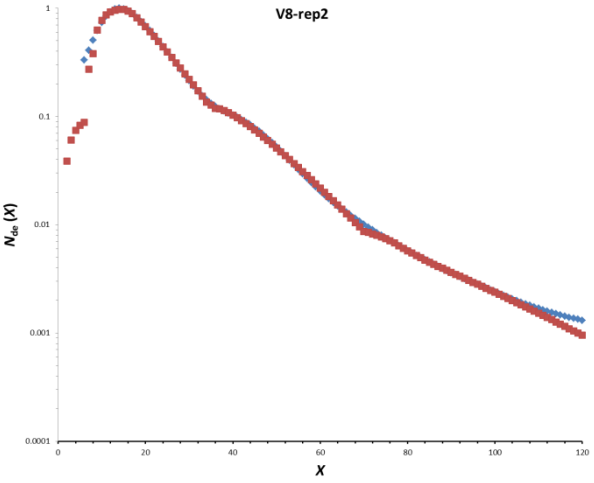
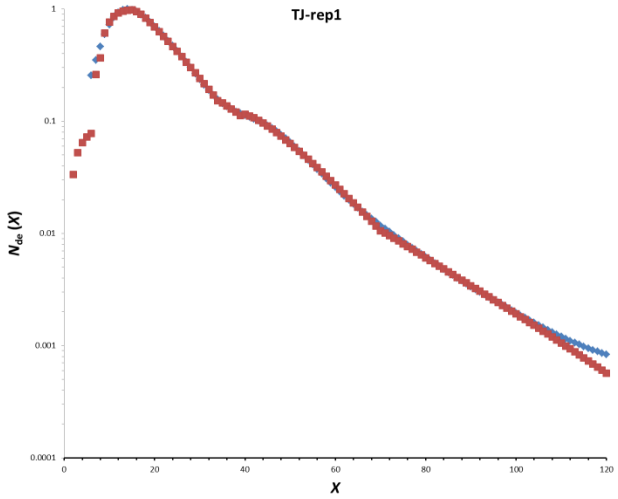

V8-rep2
$N_{de}(X)$
X


TJ-rep1
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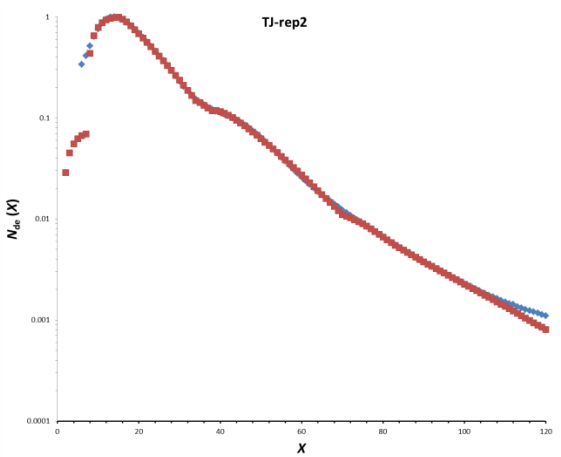
X


TJ-rep2
$N_{de}(X)$
X

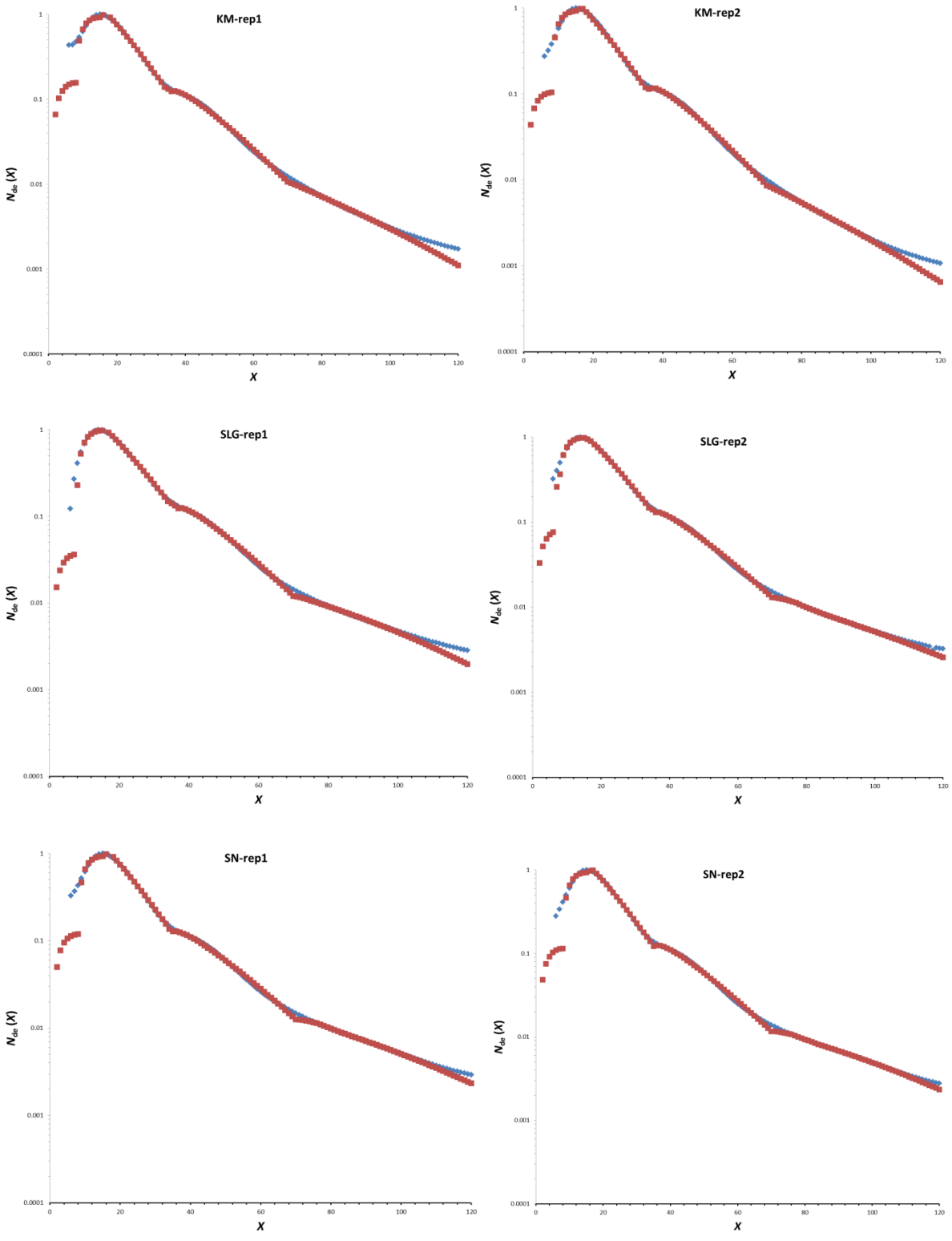


Figure S 2.1 Fitting SEC number CLDs in Wu-Gilbert Model. Chain length distribution of the SEC experiment (in blue) and the biosynthesis based model fit (in red).

Chapter 3 The molecular structural features controlling stickiness in cooked rice, a major palatability determinant

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Chapter abstract: The stickiness of cooked rice is important for eating quality and consumer acceptance. The first molecular understanding of stickiness is obtained from leaching and molecular structural characteristics during cooking. Starch is a highly branched glucose polymer. We find (i) the molecular size of leached amylopectin is 30 times smaller than that of native amylopectin while (ii) that of leached amylose is 5 times smaller than that of native amylose, (iii) the chain-length distribution (CLD: the number of monomer units in a chain on the branched polymer) of leached amylopectin is similar to native amylopectin while (iv) the CLD of leached amylose is much narrower than that of the native amylose), and (v) mainly amylopectin, not amylose, leaches out of the granule and rice kernel during cooking. Stickiness is found to increase with decreasing amylose content in the whole grain, and, in the leachate, with increasing total amount of amylopectin, the proportion of short amylopectin chains, and amylopectin molecular size. Molecular adhesion mechanisms are put forward to explain this result. This molecular structural mechanism provides a new tool for rice breeders to select cultivars with desirable palatability by quantifying the components and molecular structure of leached starch.

3.1 Introduction

Rice is a major staple food world-wide. Consumer preferences are shifting towards better-quality rice, particularly towards varieties with good eating quality (Calingacion et al., 2014). Rice texture is of prime importance to eating quality and consumer acceptance. Texture is a multi-faceted sensory property, with hardness and stickiness as the most commonly determined and discriminable textural properties of cooked rice (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016b; Patindol, Gu & Wang, 2010). Rice is the only major cereal that is most often consumed in the form of whole grain after cooking. In addition to sensory evaluation by human panels, textural properties of cooked rice are commonly evaluated by texture profile analysis (TPA) with a textural analyser (Cameron & Wang, 2005; Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a). TPA is a technique that has been extensively employed to mechanically and geometrically characterize food materials. The technique involves measuring the mechanical response during a double compression, which attempts to mimic the first and second bites of a food. For cooked rice, the two most meaningful parameters derived from TPA are hardness (the force required to attain a given deformation) and adhesiveness (a quantity that simulates the work required to overcome the attractive forces between the surface of the sample and the surface of the probe with which the same comes into contact) (Friedman, Whitney & Szczesniak, 1963).

Cooked rice texture is affected by a wide range of factors, such as the amylose content (Bhattacharya & Juliano, 1985), postharvest processing (Champagne et al., 1998) and cooking method (Crowhurst & Creed, 2001). For example, the method used to cook rice can vary between different regions, and is often specific to a varietal type (Champagne et al., 2010; Crowhurst & Creed, 2001). South and East Asians always cook rice in a rice cooker with using a particular ratio of water (the absorption method); Indians prefer cooking rice by boiling it in excess water, and Americans like cooking rice in large amounts of water which is then drained. The absorption method with controlled volumes of water is applied in this study.

Starch structure has an important role in rice texture (Cameron & Wang, 2005; Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a; Ong & Blanshard, 1995a; Radhika Reddy, Zakiuddin Ali & Bhattacharya, 1993). Starch, the main component of rice grains (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a), is a branched glucose polymer comprising two types of molecules: amylopectin and amylose. Amylopectin molecules are highly branched with a vast number of short branches and relatively large molecular weights, ~$10^{7–8}$, whereas

amylose has a smaller molecular weight (~$10^{5–6}$) and a few long branches (Gilbert, Witt & Hasjim, 2013). Starch biosynthesis is a complex pathway controlled by at least four different classes of enzymes: ADP-glucose pyrophosphorylase (AGPase), starch synthases (SSs), starch branching enzymes (SBE), and debranching enzyme (DBE). The biosynthesis of amylose is mostly controlled by granule-bound starch synthase (GBSS) while that of amylopectin is more complex, involving the combined actions of SS, SBE, and DBE (Wang, Henry & Gilbert, 2014). Amylose content was, since the mid-1980s, considered to be the most important determinant of the hardness of cooked rice (Bhattacharya & Juliano, 1985). In the mid-1990s, it was proposed that hardness is more dependent on the long amylopectin chains (Ong & Blanshard, 1995a; Radhika Reddy, Zakiuddin Ali & Bhattacharya, 1993). Based on the significant role of amylose in determining the hardness of cooked rice, a set of different physicochemical methods has been developed to measure rice hardness, such as the starch-iodine blue value (Roberts, Potter, Kester & Keneaster, 1954), Brabender viscogram (Bhattacharya & Sowbhagya, 1978), alkali spreading value (Bhattacharya, Sowbhagya & Swamy, 1982) and gel consistency (Cagampang, Perez & Juliano, 1973). In previous work, we found that the molecular fine structure of amylose, both the molecular size and chain-length distribution, are also significant determinants of the hardness of cooked rice (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a).

In contrast to hardness, stickiness between rice grains is less commonly investigated, and the mechanism of rice stickiness is unclear, even though stickiness between rice grains is the key requirement for sushi, which is a very popular food. Stickiness has previously been related to grain length, with short grains being usually thought of as sticky and the long grains as not (Mossman, Fellers & Suzuki, 1983). Recent studies show that stickiness is always negatively correlated with amylose content and hardness, i.e. high-amylose rice is usually harder and less sticky while low-amylose rice is softer and sticky (Cameron & Wang, 2005; Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a, b; Patindol, Gu & Wang, 2010). Nevertheless, rice cultivars with similar amylose contents can still display different stickiness (Ayabe, Kasai, Ohishi & Hatae, 2009). Very few publications address the structural reasons for stickiness of cooked rice. Patindol, Gu and Wang (2010) suggested that the amylose-amylopectin ratio of the leached materials during rice cooking may be the main indicator of cooked rice hardness and stickiness. Ayabe, Kasai, Ohishi and Hatae (2009) compared the stickiness of two rice cultivars with similar amylose content, Nipponbare (Japonica rice) and Khao Dawk Mali

(Indica rice), and suggested that the difference in the amount of leached materials from the surface of cooked rice contributed to the differences in stickiness. Since the stickiness measured by a texture analyser actually reflects the adhesiveness between interfaces i.e. the surface between rice kernel and TPA probe, this indicates that physical and chemical characteristics of the surface materials (the leached materials during cooking) are likely to be a major determinant of the stickiness between rice grains.

Using a set of rice cultivars differing in terms of amylose content, the objectives of this study are: 1) to identify and characterize the amounts and molecular structural features (both chain-length distribution (CLD) and molecular size, measured by size-exclusion chromatography (SEC, also termed GPC or HPLC-SEC, where the size separation parameter is the hydrodynamic radius *R*h) of the leached starch; and 2) to devise mechanistic reasons for any differences in terms of leaching characteristics and molecular structural features of leached starch.

3.2 Materials and methods

3.2.1 Materials

Twelve varieties of rice were selected with a wide range with known phenotypes and genotypes for quality traits (**Table 3.1**). After harvesting, all rice samples were dehulled in a dehusker (Otake, Aichi, Japan), polished to yield rice with the same whiteness value in a commercial mill (FASCO, VIC, Australia), and then stored in self-sealing plastic bags in a refrigerator prior to analyses.

Protease from *Streptomyces griseus* (type XIV), and LiBr (ReagentPlus) were purchased from Sigma-Aldrich Pty. Ltd. (Castle Hill, Australia). Isoamylase (from *Pseudomonas sp.*) and a D-glucose (glucose oxidase/peroxidase; GODOP) assay kit were purchased from Megazyme International, Ltd. (Wicklow, Ireland). A series of pullulan standards with peak molecular weights ranging from 342 to 2.35 $\times 10^6$ were from Polymer Standards Service (PSS) GmbH (Mainz, Germany). Dimethyl sulfoxide (DMSO, GR grade for analysis) was from Merck Co. Inc. (Kilsyth, Australia). All other chemicals were reagent-grade and used as received.

3.2.2 Rice cooking

Before cooking, residual bran and other adhering powders were removed from white rice kernels with an aspirating device. A 10-g sample of white rice was placed in a 100 mL beaker,

and distilled water was added to the rice to give a rice-to-water weight ratio of 1:1.6. Thereafter, the beaker was sealed with aluminium foil, placed on a steaming tray, and cooked in a household rice cooker (Kambrook Rice Express, VIC, Australia) for 30 min.

3.2.3 Texture profile analysis (TPA)

After cooking and cooling to room temperature, a 1-g subsample of cooked rice grains was weighed and placed as a single layer of grains on the base plate. A two-cycle, force-versus-distance compression program was used for measurements with a TA.XT-Plus Texture analyser with a 35 mm cylindrical probe attachment (Stable Micro Systems Ltd., Surrey, UK). The probe descended at a speed of 1 mm/s, returned, and then the compression cycle was repeated. Compression was set to 40% strain to avoid destroying the rice grain. For each of 3 cooking replicates, texture measurements were conducted 6 times on the 1-g subsample of cooked rice grains. Stickiness between grains was recorded as the area of the negative force curve.

3.2.4 Extraction of leached materials

A sample of white rice (10 g) was cooked as described above. The leached materials on the surface of the cooked rice were extracted by rinsing with 100 mL of hot deionized water (~95 ℃) with very gentle stirring using a glass rod for 5 – 10 s before filtering through a 250 μm sieve. The rinsing procedure was repeated again with 50 mL of hot deionized water. Both the washed kernels and the rinsing water were retained. The rinsed rice kernels were cooled and used to measure stickiness again by TPA. The water was frozen immediately using liquid nitrogen, and then freeze-dried for storage and further analysis. The total weight of the leached materials was recorded after freeze-drying.

3.2.5 Composition analysis of leached materials

Total starch content of leached materials was measured using a Megazyme total starch (AA/AMG) assay kit following a method described elsewhere (Zou, Sissons, Gidley, Gilbert & Warren, 2015). The protein content of leached materials was determined using a BCA Protein Assay Kit (Pierce).

3.2.6 Molecular size distributions of both whole-grain starch and leached starch molecules

The structure of extracted whole starch and leached starch molecules was characterized by SEC using an Agilent 1100 Series SEC system (Agilent Technologies, Waldbronn, Germany) equipped with GRAM 30 and 3000 analytical columns (PSS) and a refractive index (RI) detector (RID-10A, Shimadzu Corp., Kyoto, Japan), following a method described elsewhere (Cave, Seabrook, Gidley & Gilbert, 2009; Liu, Halley & Gilbert, 2010). The molecular size distribution of branched starch was plotted as the SEC weight distribution, $w_{br}(\log R_h)$. For branched starch molecules, as for any branched polymer, there is no unique relation between size and the molecular weight. For the debranched samples, which are linear, the relation between *R*h and molecular weight *M* was obtained as follows. The assumption of universal calibration for SEC is that the elution time of the analyte depends only on its R_h and not on its structure, whence one has for two linear polymers, a sample and a standard, the relation:

$$K_{\text{standard}} M^{\alpha(\text{standard})+1} = K_{\text{sample}} M^{\alpha(\text{sample})+1} \quad (1)$$

where *K* and α are the Mark-Houwink parameters for the polymer, solvent and temperature being used. Pullulan standards with known peak molecular weights were used for calibration to obtain a relationship between SEC elution volume and R_h of starch molecules following the Mark-Houwink equation:

$$V\text{h} = 4/3\ \pi\ R\text{h}3 = \frac{2}{5}\frac{K1 + \alpha}{N\text{A}} \quad (2)$$

Here N_A is Avogadro's constant. The Mark-Houwink parameters *K* and α of pullulan in DMSO/LiBr solution at 80 ℃ are 2.424×10^{-4} dL g^{-1} and 0.68, respectively(Cave, Seabrook, Gidley & Gilbert, 2009).

3.2.7 Starch debranching and measurement of CLD of debranched starch by SEC

The extracted starch (~4 mg) was dissolved in 0.9 mL of deionized water and then mixed with 2.5 μL isoamylase (1000 U mL^{-1}), 0.1 mL acetate buffer solution (0.1 M, pH 3.5), and 5 μL sodium azide solution (0.04 g mL^{-1}). The mixture was incubated at 37 ℃ for 3 h. The debranched starch suspension was then heated in a water bath at 80 ℃ for 2 h after being neutralized with 0.1 M NaOH solution, and then freeze-dried overnight. The dried debranched starch was dissolved in DMSO/LiBr (0.5%) solution for SEC analysis.

To obtain SEC distributions of debranched starch, GRAM 100 and GRAM 1000 columns (PSS) were used, with the same pullulan standards and procedure as that used to calibrate the SEC for whole branched molecules. The SEC weight distribution, $w(\log X)$, obtained from the

DRI signal was plotted against X (degree of polymerization DP), with X being determined using the Mark-Houwink relationship (see Equation 1) and with $M = 162.2(X–1)+18.0$ (162.2 is the molecular weight of the anhydroglucose monomeric unit and 18.0 is that of the additional water in the end groups); K and α for linear starch chains in the eluent of DMSO/LiBr at 80 ℃ are 1.5×10^{-4} dL g^{-1} and 0.743, respectively(Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a). For a linear polymer (such as debranched starch), the number distribution (obtained by debranching), $N\mathrm{de}(X)$, is related to the corresponding weight distribution by (Castro, Ward, Gilbert & Fitzgerald, 2005):

$$w(\log X) = X^2 N_{\mathrm{de}}(X) \quad (3)$$

The amylose content of all rices was determined from the SEC weight distributions of debranched starch following the procedure described by Syahariza, Sar, Hasjim, Tizzotti and Gilbert (2013). This method has been shown to be more accurate than the iodine colorimetric method (Vilaplana, Hasjim & Gilbert, 2012).

3.2.8 Statistical analysis

For each structural measurement, duplicate analyses were performed for each sample. All data were reported as mean ± standard deviation (SD) using analysis of variance (ANOVA) with Tukey's pairwise comparisons. Significant differences of the mean values were determined at $p < 0.05$. The textural measurements were analysed in duplicate for each sample. One-way analysis of variance (ANOVA) and both Pearson and Spearman rank correlation methods were carried out using SPSS V. 16.0 software (SPSS Inc., Chicago, IL). The means of duplicated measurements were used for the correlation analysis.

3.3 Results

3.3.1 Stickiness of freshly cooked rice with and without hot-water washing

The stickiness of the grains from 12 freshly cooked rice varieties displays significant differences. KN and HMN, both waxy rices, are the two stickiest, whereas SN and SLG, both high-amylose rices, show extremely low stickiness (**Fig. 3.1**). This is consistent with our previous results that stickiness is always negatively correlated with amylose content (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a, b). After hot-water washing, most varieties, except SN and SLG, show similar and reduced stickiness values. As displayed in **Table 3.1**,

the relative stickiness loss ranges from 65 to 86%, and both the absolute and relative amounts of stickiness loss are reduced with increasing amylose content (**Table 3.1**).

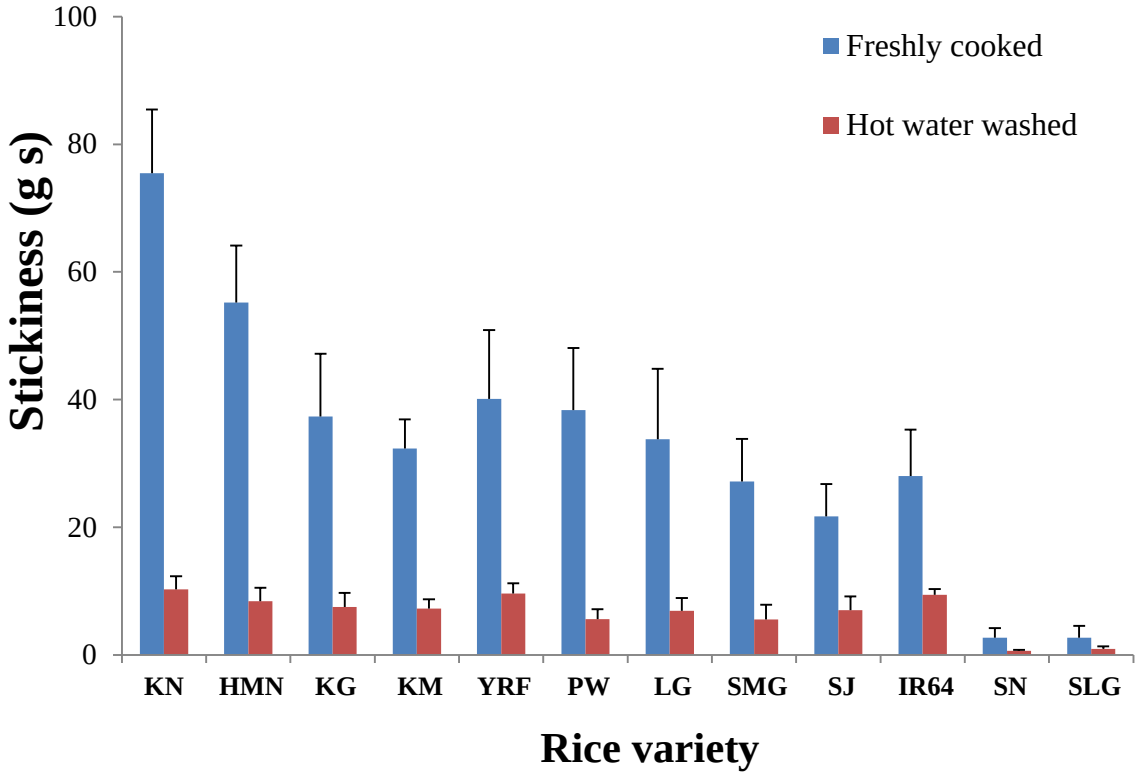

Figure 3.1 Stickiness of all rice samples measured from TPA.

Table 3.1 Parameters of the stickiness and the leaching characteristics of all rice varieties.

Rice variety	Abbreviation code	Country of origin	Amylose content	Stickiness (g·s)		Stickiness Loss	
				Freshly cooked	Hot-water washed	Absolute (g·s)	Relative
Khao Niao	KN	Thailand	3.3 ± 0.1 % a	75.4 ± 22.3 e	10.3 ± 2.1 d	65	86%
Hom mali Niaw	HMN	Australia	2.6 ± 0.3 % a	55.2 ± 8.9 d	8.4 ± 2.1 b-d	47	85%
Kangaroo	KG	Australia	19.6 ± 0.9 % b	37.4 ± 9.8 b,c	7.5 ± 2.2 b-d	30	80%
Kyeema	KM	Australia	19.5 ± 0.5 % b	32.4 ± 4.6 b,c	7.3 ± 1.5 a,b	25	78%
YRF209	YRF	Australia	22.3 ± 0.2 % b,c	40.1 ± 10.7 c,d	9.6 ± 1.6 c,d	31	76%
Pandan Wangi	PW	Australia	20.4 ± 1.9 % b	38.4 ± 9.7 c	5.6 ± 1.6 b	33	85%
Langi	LG	Australia	21.7 ± 0.1 % b,c	33.8 ± 11.0 b,c	6.9 ± 1.9 a,b	27	79%
Sunrice Medium Grain	SMG	Australia	21.8 ± 0.9 % b,c	27.2 ± 6.7 b,c	5.6 ± 2.3 b	22	80%
Sunrice Jasmine	SJ	Australia	21.2 ± 1.4 % b	21.7 ± 5.0 b	7.0 ± 2.2 a,b	15	68%
IR64	IR64	Australia	24.9 ± 0.5 % c	28.1 ± 7.3 b,c	9.4 ± 0.9 c,d	19	66%
Swarna	SN	India	31.2 ± 0.1 % d	2.7 ± 1.5 a	0.6 ± 0.2 a	2	76%
Sunrice Long Grain	SLG	Thailand	32.0 ± 0.2 % d	2.7 ± 1.8 a	0.9 ± 0.4 a	2	65%

	Components content (%) in the leachate		Amylose content of the leached starch	Total solids of the leachate (mg/ g rice kernel)	Components weight in the leachate (mg/ g rice kernel)		
	Starch	Protein			Total starch	Total amylose	Total protein
KN	89.64 ± 0.00 a,b	2.96 ± 0.00 b	0.72 ± 0.00 a	43.7 ± 0.00 g	33.7 ± 0.00 f	0.2 ± 0.00 a	1.1 ± 0.00 d
HMN	89.67 ± 0.64 a,b	2.01 ± 0.06 a,b	1.73 ± 0.17 a	55.1 ± 2.79 h	42.5 ± 2.45 g	0.7 ± 0.12 a,b	1.0 ± 0.08 c,d
KG	91.23 ± 3.36 a,b	2.58 ± 1.17 a,b	15.52 ± 0.34 b,c	23.0 ± 0.00 b,c	18.1 ± 0.67 b,c	2.8 ± 0.16 c,d	0.5 ± 0.23 b,c
KM	91.30 ± 5.72 a,b	1.91 ± 0.04 a,b	10.26 ± 2.21 b	25.2 ± 1.19 c,d	19.8 ± 2.17 b-d	2.1 ± 0.66 b,c	0.4 ± 0.01 b,c
YRF	92.39 ± 1.26 b	1.55 ± 0.27 a,b	14.66 ± 0.69 b,c	27.7 ± 1.19 d,e	22.0 ± 1.24 c-e	3.2 ± 0.33 c,d	0.4 ± 0.05 a,b
PW	92.65 ± 4.36 b	2.24 ± 0.07 a,b	11.63 ± 1.74 b,c	33.2 ± 0.35 f	26.5 ± 0.97 e	3.1 ± 0.35 c,d	0.6 ± 0.01 b,c
LG	91.76 ± 0.63 a,b	2.13 ± 0.59 a,b	17.07 ± 0.44 b,c	30.3 ± 0.08 e,f	23.9 ± 0.10 d,e	4.1 ± 0.12 d,e	0.6 ± 0.16 b,c
SMG	90.08 ± 0.30 a,b	2.57 ± 0.32 a,b	17.93 ± 4.89 c	23.8 ± 0.85 b-d	18.4 ± 0.60 b,c	3.3 ± 0.79 c,d	0.5 ± 0.08 b,c
SJ	81.18 ± 2.83 a	2.67 ± 0.16 a,b	13.04 ± 0.57 b,c	22.7 ± 0.45 b,c	15.9 ± 0.87 a,b	2.1 ± 0.02 b,c	0.5 ± 0.04 b,c
IR64	90.97 ± 2.57 a,b	1.16 ± 0.11 a	18.17 ± 1.81 c	26.0 ± 0.59 c-e	20.3 ± 0.11 b-d	3.7 ± 0.35 c,d	0.3 ± 0.02 a
SN	86.40 ± 0.54 a,b	2.02 ± 0.27 a,b	42.61 ± 0.00 d	15.8 ± 1.46 a	11.7 ± 1.16 a	5.34 ± 0.00 e	0.3 ± 0.06 a
SLG	90.93 ± 2.43 a,b	1.96 ± 0.03 a,b	44.05 ± 2.36 d	20.3 ± 0.58 b	15.9 ± 0.88 a,b	7.0 ± 0.76 f	0.3 ± 0.00 a,b

*Absolute value of stickiness is calculated by the stickiness of freshly cooked rice minus that of hot-water washed rice; The absolute value of stickiness loss relative to the stickiness of the freshly cooked rice.

3.3.2 Composition of leached materials

The composition of the leached materials is presented in **Table 3.1**. The total starch content ranges from 81.2 to 92.6%, the protein content from 1.2 to 3.0%, and amylose content of the leached starch ranges from nearly 0 to 44%. Both leached starch and protein content show little significant difference while leached amylose content is significantly different. Rices with higher amylose content leach more amylose. The total solids of leached materials range from 15 to 55 mg per initial weight (g) rice kernel, making total weight of leached starch and protein significantly different between cultivars and also showing that high-amylose rices leached less material than waxy or low-amylose rices.

3.3.3 The structural characterization of leached starch

Fig. 3.2 presents typical SEC weight distributions of branched starch molecules. As shown in **Fig. 3.2a** and described elsewhere (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a; Syahariza, Sar, Hasjim, Tizzotti & Gilbert, 2013), the fully branched distributions of native grain starch show two populations of α-glucans: amylose ($R_h \leq 100$ nm) and amylopectin ($R_h >$ 100 nm). The elution pattern of the two waxy varieties indicates that there is some co-elution of small amylopectin molecules and large amylose molecules. Another small peak at R_h ~1 nm may be residual proteins, due to the incomplete hydrolysis by protease during the starch extraction procedure. For the leached starches (**Fig. 3.2b**), the molecular size distributions are over a significantly smaller range (1 ~ 100 nm) than those of native grain starch (1 ~ 1000 nm), with almost none of the very large molecules present in the leachate. There are two populations of molecules in the leached starches, at R_h ~1 nm and ~10 nm. The leached amylose and amylopectin were not clearly separated, which may either be because their ranges overlapped in size, or the limitations of SEC separation for the set-up used here. As mentioned above, waxy rice leaches mainly amylopectin, but high-amylose rice leaches significantly higher proportions of amylose. **Table 3.2** shows the average molecular size of amylopectin and amylose, $\bar{R}_h$, as defined elsewhere (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a). The $\bar{R}_{h,Ap}$ of grain amylopectin is about 30 times higher than that of leached amylopectin while the $\bar{R}h_{,Am}$ of grain amylose is about 5 times higher than that of leached amylose.

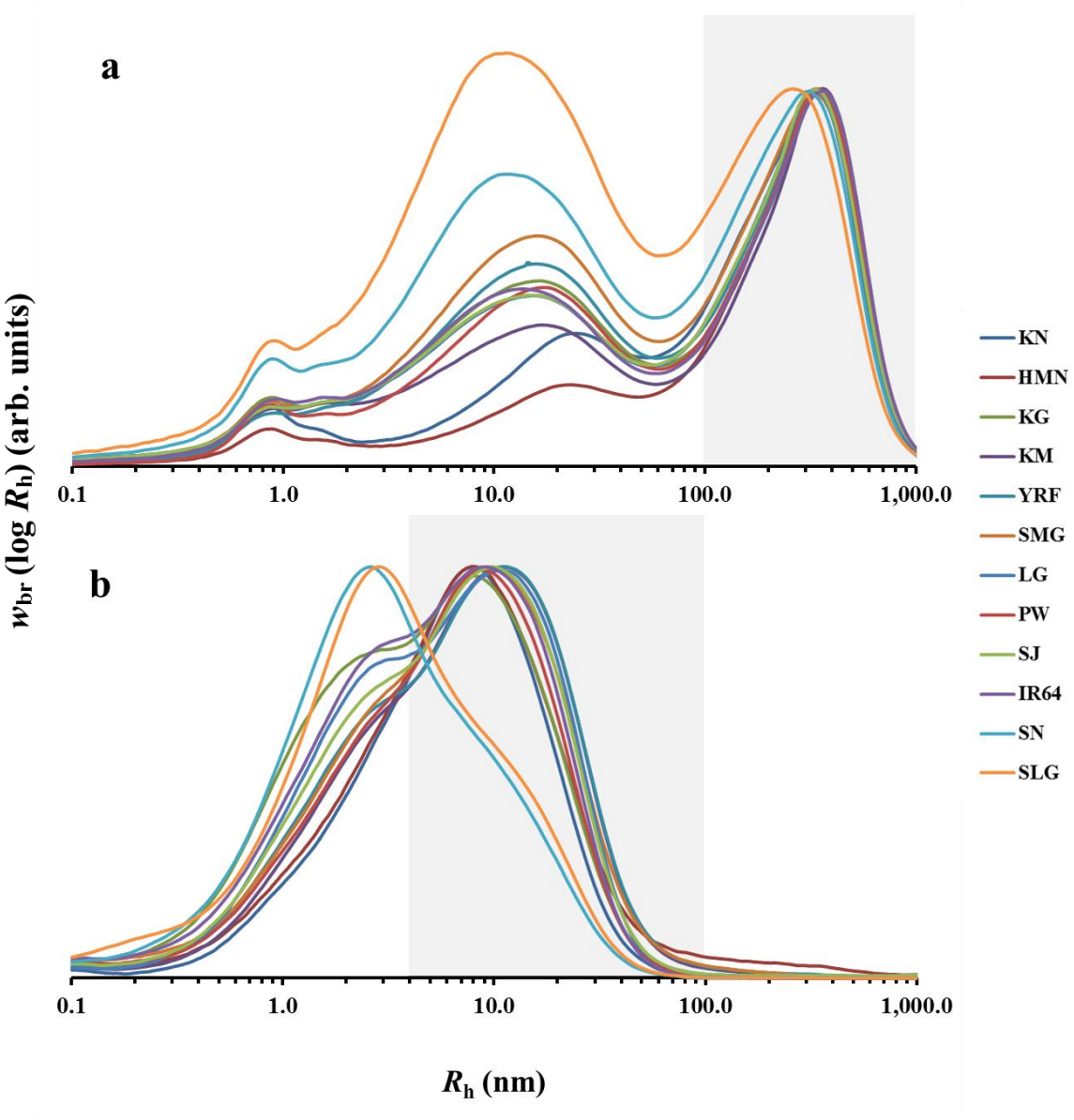

Figure 3.2 SEC weight distributions of branched starch molecules, $w_{br}(\log R_h)$, normalized to the highest peak. a) Weight distributions for native grain starch. b) weight distributions for leached starch. The grey area denotes the R_h range of amylopectin in native grain and leachate, respectively.

Table 3.2 Starch molecular parameters extracted from SEC for all native grain starches and leached starches.

Rice variety & treatment		Amylopectin								
		$\overline{R_{h_{AP}}}$	X_{Ap1}	X_{Ap2}	h_{Ap2}	6<X≤12	12<X≤24	24<X≤36	36<X≤100	$\bar{X}_{AP}$
Grain starch	KN	223.5 ±4.4 [b,c]	16.1 ±0.0 [a-c]	40.9 ±0.0 [e]	0.70 ±0.00 [c-f]	24.3 ±0.0% [a-e]	37.4 ±0.0% [c-f]	16.9 ±0.0% [d-h]	21.4 ±0.0% [a-e]	14.6 ±0.0 [a]
	HMN	242.0 ±0.9 [c]	15.2 ±0.8 [a,b]	40.4 ±1.7 [d,e]	0.67 ±0.01 [b-f]	26.8 ±3.1% [c-e]	37.1 ±1.2% [c-f]	15.6 ±0.6% [a-f]	20.5 ±1.2% [a-e]	13.6 ±1.6 [a]
	KG	241.8 ±10.7 [c]	17.2 ±1.5 [b,c]	39.0 ±0.7 [a-e]	0.76 ±0.08 [f-h]	22.2 ±2.5% [a-c]	36.2 ±1.0% [b-e]	17.9 ±1.3% [h]	23.7 ±2.2% [e,f]	14.0 ±2.2 [a]
	KM	248.5 ±3.4 [c]	16.3 ±0.3 [a-c]	40.0 ±0.7 [b-e]	0.63 ±0.00 [a-d]	22.6 ±1.0% [a-d]	39.7 ±0.4% [c-f]	16.0 ±0.2% [b-f]	21.8 ±0.4% [a-e]	13.9 ±0.4 [a]
	YRF	235.4 ±3.8 [c]	15.4 ±0.3 [a,b]	40.0 ±0.2 [b-e]	0.60 ±0.01 [a,b]	26.9 ±1.4% [c-e]	38.6 ±0.6% [c-f]	15.1 ±0.3% [a-c]	19.3 ±0.5% [a-c]	13.8 ±1.6 [a]
	PW	224.9 ±13.5 [b,c]	15.4 ±0.3 [a,b]	40.2 ±0.5 [c-e]	0.61 ±0.00 [a-c]	26.1 ±1.1% [c-e]	38.3 ±0.2% [c-f]	15.2 ±0.1% [a-c]	20.4 ±0.8% [a-e]	14.5 ±0.2 [a]
	LG	232.5 ±17.0 [c]	16.3 ±0.0 [a-c]	38.3 ±1.2 [a-e]	0.67 ±0.06 [b-f]	22.5 ±0.5% [a-d]	39.1 ±1.4% [d-f]	17.2 ±0.8% [e-h]	21.2 ±1.2% [a-e]	14.6 ±0.6 [a]
	SMG	241.2 ±11.9 [c]	16.5 ±0.0 [a-c]	38.0 ±0.2 [a-e]	0.73 ±0.01 [d-g]	23.8 ±0.2% [a-e]	36.8 ±0.8% [b-f]	17.7 ±0.2% [g,h]	21.8 ±0.8% [a-e]	13.2 ±2.6 [a]
	SJ	233.4 ±10.8 [c]	16.1 ±0.0 [a-c]	39.2 ±0.5 [a-e]	0.65 ±0.01 [a-d]	22.9 ±0.2% [a-d]	39.4 ±0.1% [e,f]	16.6 ±0.2% [c-h]	21.2 ±0.3% [a-e]	14.2 ±0.5 [a]
	IR64	244.6 ±14.4 [c]	16.0 ±0.1 [a-c]	40.9 ±0.5 [e]	0.61 ±0.01 [a-c]	23.6 ±0.2% [a-e]	39.6 ±0.0% [f]	15.0 ±0.1% [a-c]	21.8 ±0.1% [a-e]	14.7 ±0.2 [a]
	SN	210.0 ±11.9 [b,c]	16.8 ±0.4 [a-c]	39.8 ±1.0 [b-e]	0.75 ±0.02 [e-g]	20.5 ±0.9% [a,b]	36.3 ±0.1% [b-f]	17.3 ±0.8% [f-h]	25.9 ±0.2% [f,g]	14.6 ±1.3 [a]
	SLG	190.4 ±2.4 [b]	17.5 ±1.1 [c]	39.8 ±0.0 [b-e]	0.85 ±0.03 [h]	20.5 ±0.6% [a,b]	33.5 ±0.4% [b]	17.7 ±0.4% [h]	28.3 ±0.6% [g]	15.1 ±0.7 [a]
Leached starch	KN	8.7 ±0.1 [a]	16.0 ±0.1 [a-c]	39.5 ±0.0 [a-e]	0.65 ±0.00 [a-e]	26.4 ±0.0% [c-e]	37.9 ±0.2% [c-f]	16.7 ±0.1% [c-h]	19.1 ±0.3% [a,b]	11.0 ±1.2 [a]
	HMN	9.1 ±0.2 [a]	15.7 ±0.1 [a-c]	39.0 ±0.2 [a-e]	0.63 ±0.01 [a-d]	26.9 ±0.1% [c-e]	38.2 ±0.2% [c-f]	16.5 ±0.0% [c-h]	18.5 ±0.1% [a]	13.2 ±0.1 [a]
	KG	8.6 ±0.1 [a]	15.9 ±0.0 [a-c]	38.7 ±0.2 [a-e]	0.65 ±0.01 [a-e]	24.7 ±0.0% [b-e]	36.4 ±0.1% [b-f]	16.0 ±0.1% [b-g]	22.8 ±0.0% [c-f]	13.1 ±0.8 [a]
	KM	8.9 ±0.1 [a]	16.0 ±0.1 [a-c]	38.5 ±0.0 [a-e]	0.61 ±0.02 [a-c]	24.7 ±0.3% [b-e]	37.5 ±1.1% [c-f]	15.6 ±0.0% [a-e]	22.1 ±0.8% [b-e]	11.2 ±2.6 [a]
	YRF	9.0 ±0.1 [a]	15.4 ±0.0 [a,b]	37.0 ±0.2 [a-c]	0.60 ±0.02 [a,b]	27.3 ±0.9% [d,e]	37.4 ±0.9% [c-f]	15.4 ±0.0% [a-d]	19.9 ±1.7% [a-d]	11.7 ±0.4 [a]
	PW	9.1 ±0.0 [a]	15.5 ±0.4 [a-c]	38.5 ±0.0 [a-e]	0.63 ±0.01 [a-c]	27.1 ±1.0% [d,e]	36.8 ±1.8% [b-f]	15.9 ±0.2% [b-f]	20.2 ±1.1% [a-e]	10.7 ±3.0 [a]
	LG	8.5 ±0.0 [a]	15.1 ±0.4 [a]	36.7 ±1.6 [a,b]	0.57 ±0.00 [a]	27.2 ±1.7% [d,e]	37.5 ±0.9% [c-f]	14.8 ±0.3% [a,b]	20.5 ±0.5% [a-e]	11.0 ±1.4 [a]
	SMG	8.9 ±0.2 [a]	14.9 ±0.4 [a]	36.4 ±2.0 [a]	0.63 ±0.01 [a-c]	27.9 ±1.3% [d,e]	35.3 ±1.1% [b,c]	15.1 ±0.1% [a-c]	21.7 ±0.1% [a-e]	11.8 ±0.4 [a]
	SJ	8.7 ±0.0 [a]	15.2 ±0.5 [a,b]	37.5 ±0.9 [a-d]	0.58 ±0.00 [a,b]	27.1 ±1.9% [d,e]	38.5 ±1.1% [c-f]	15.2 ±0.3% [a-c]	19.2 ±0.5% [a,b]	11.9 ±1.3 [a]
	IR64	8.6 ±0.3 [a]	15.6 ±0.5 [a-c]	38.0 ±1.2 [a-e]	0.64 ±0.01 [a-d]	25.5 ±1.2% [c-e]	35.9 ±1.4% [b-d]	15.3 ±0.2% [a-c]	23.4 ±0.4% [d-f]	11.3 ±1.4 [a]
	SN	7.5 ±0.1 [a]	15.8 ±0.0 [a-c]	40.9 ±0.0 [e]	0.80 ±0.00 [g,h]	19.6 ±0.0% [a]	29.2 ±0.0% [a]	14.0 ±0.0% [a]	37.3 ±0.0% [h]	11.5 ±0.0 [a]
	SLG	7.6 ±0.0 [a]	15.9 ±0.8 [a-c]	40.3 ±0.2 [c-e]	0.85 ±0.04 [h]	20.5 ±1.6% [a,b]	27.7 ±0.5% [a]	14.2 ±0.2% [a]	37.6 ±1.9% [h]	11.5 ±0.7 [a]

Rice variety & treatment		Amylose				
		$\overline{R_{\mathrm{h_{AM}}}}$	AM content	100<X≤1000	1000<X≤20000	$\bar{X}_{\mathrm{AM}}$
Grain starch	KN	-	-	-	-	-
	HMN	-	-	-	-	-
	KG	10.6 ± 0.1 [b-e]	19.6 ± 0.9% [e-g]	13.8 ± 0.5% [a-d]	5.8 ± 0.4% [b]	701.5 ± 23.9 [c-e]
	KM	10.7 ± 0.1 [c-e]	19.5 ± 0.5% [e-g]	13.8 ± 0.4% [a-d]	5.7 ± 0.1% [b]	651.9 ± 2.4 [c,d]
	YRF	10.8 ± 0.1 [d-e]	22.3 ± 0.2% [g,h]	14.7 ± 0.1% [b-d]	7.6 ± 0.2% [c]	805.4 ± 11.3 [e,f]
	PW	10.9 ± 0.0 [d-e]	20.4 ± 1.9% [f-h]	13.9 ± 1.2% [a-d]	6.5 ± 0.7% [b,c]	727.3 ± 13.4 [d-f]
	LG	10.5 ± 0.1 [b-e]	21.7 ± 0.0% [g,h]	14.3 ± 0.3% [b-d]	7.4 ± 0.4% [c]	821.3 ± 31.3 [f]
	SMG	11.2 ± 0.1 [e]	21.8 ± 1.0% [g,h]	14.9 ± 0.6% [b-d]	6.9 ± 0.4% [b,c]	777.4 ± 32.9 [e,f]
	SJ	10.5 ± 0.2 [b-e]	21.2 ± 1.4% [f-h]	14.5 ± 1.6% [b-d]	6.7 ± 0.2% [b,c]	768.6 ± 96.0 [e,f]
	IR64	10.3 ± 0.4 [b-d]	24.9 ± 0.5% [h]	17.1 ± 0.2% [c,d]	7.8 ± 0.3% [c]	699.6 ± 9.0 [c-e]
	SN	10.1 ± 0.1 [b,c]	31.2 ± 0.1% [i]	23.7 ± 0.6% [e]	7.5 ± 0.5% [c]	609.0 ± 37.6 [b,c]
	SLG	10.0 ± 0.1 [b]	32.0 ± 0.2% [i]	26.4 ± 0.5% [e]	5.6 ± 0.6% [b]	498.2 ± 25.6 [b]
Leached starch	KN	-	-	-	-	-
	HMN	-	-	-	-	-
	KG	2.9 ± 0.5 [a]	15.5 ± 0.3% [b-e]	15.3 ± 0.3% [b-d]	0.3 ± 0.1% [a]	205.6 ± 8.6 [a]
	KM	2.6 ± 0.1 [a]	10.3 ± 2.2% [a]	9.9 ± 2.3% [a]	0.4 ± 0.1% [a]	192.2 ± 9.6 [a]
	YRF	2.8 ± 0.1 [a]	14.7 ± 0.7% [a-d]	14.6 ± 0.7% [b-d]	0.0 ± 0.0% [a]	209.9 ± 9.5 [a]
	PW	2.9 ± 0.0 [a]	11.6 ± 1.7% [a,b]	11.2 ± 1.4% [a,b]	0.5 ± 0.4% [a]	238.0 ± 15.7 [a]
	LG	2.6 ± 0.1 [a]	17.1 ± 0.4% [c-f]	17.0 ± 0.5% [c,d]	0.1 ± 0.1% [a]	204.2 ± 1.9 [a]
	SMG	2.8 ± 0.2 [a]	14.5 ± 0.0% [a-d]	14.1 ± 0.0% [a-d]	0.4 ± 0.0% [a]	209.5 ± 23.5 [a]
	SJ	2.8 ± 0.0 [a]	13.0 ± 0.6% [a-c]	12.9 ± 0.7% [a-c]	0.1 ± 0.1% [a]	218.8 ± 1.4 [a]
	IR64	2.9 ± 0.2 [a]	18.2 ± 1.8% [d-g]	17.9 ± 1.9% [d]	0.3 ± 0.1% [a]	213.1 ± 17.7 [a]
	SN	2.8 ± 0.2 [a]	42.6 ± 0.0% [j]	42.0 ± 0.0% [f]	0.6 ± 0.0% [a]	220.3 ± 0.0 [a]
	SLG	2.7 ± 0.0 [a]	44.1 ± 2.4% [j]	44.0 ± 2.3% [f]	0.0 ± 0.0% [a]	211.5 ± 16.4 [a]

*Mean ± SD is calculated from duplicate measurements. Values with different letters in the same column are significantly different with $p < 0.05$.

Fig. 3.3 displays typical weight chain-length distributions (CLDs) of debranched starches. The components with $X \leqslant 100$ are defined as amylopectin chains, while those with $X > 100$ are defined as amylose chains (Vilaplana, Hasjim & Gilbert, 2012). For grain (**Fig. 3.3a**) and leached (**Fig. 3.3b**) starches, the weight CLDs of amylopectin show the usual features of two large amylopectin peaks (denoted AP1 and AP2, respectively). The waxy varieties also show the presence of some very long chains, with $X > 100$, which are absent in the CLD of the leached starch. As displayed in **Table 3.2**, for either native grain starch or leached starch, X_{AP1} is about DP 15 – 17 while X_{AP2} is between DP 37 – 40, showing little significant differences. However, as shown in **Fig. 3.3** and **Table 3.2**, the height of the second peak (denoted h_{AP2}) varies significantly, especially for high-amylose rices, for which h_{AP2} of both grain and leached starches are much higher than that of other rice samples. **Table 3.2** also gives a subdivision method by Hanashiro, Abe and Hizukuri (1996) to separate the amylopectin region into four categories: X = 6 – 12, 13 – 24, 25 – 36, and 37 – 100, described as short, medium, long, and very long chains respectively. Waxy rices (KN and HMN) have more short branches and fewer very long chains, whereas high-amylose rices show an opposite distribution with less short chains but more very long chains. For the amylopectin CLD comparing grain and leached starch, most rice cultivars, except high-amylose rices, do not display large variations. Compared to the amylopectin CLD of grain starch for high-amylose rices, the leached starch of high-amylose rices contains significantly less medium and long chains but more very long chains. Even though the average DP of amylopectin $\overline{X}_{Ap}$ of both grain and leached starch is not significantly different (**Table 3.2**), the amylose CLDs between grain and leached amylose are obviously different. The amylose branches of grain starch range from DP 100 to 20,000 (**Fig. 3.3a**), whereas that of leached starch just range between DP 100 and 1000 (**Fig. 3.3b** and **Table 3.2**). Even though the amount of leached amylose varies between cultivars, the average DP ($\overline{X}_{Am}$) of leached amylose is not significantly different compared to that of native amylose in the grain. Interestingly, the super-long chains seen for the two waxy varieties in the CLD of the grain starch are not present in the CLD of the leached starch for those samples (**Fig. 3.3b**).

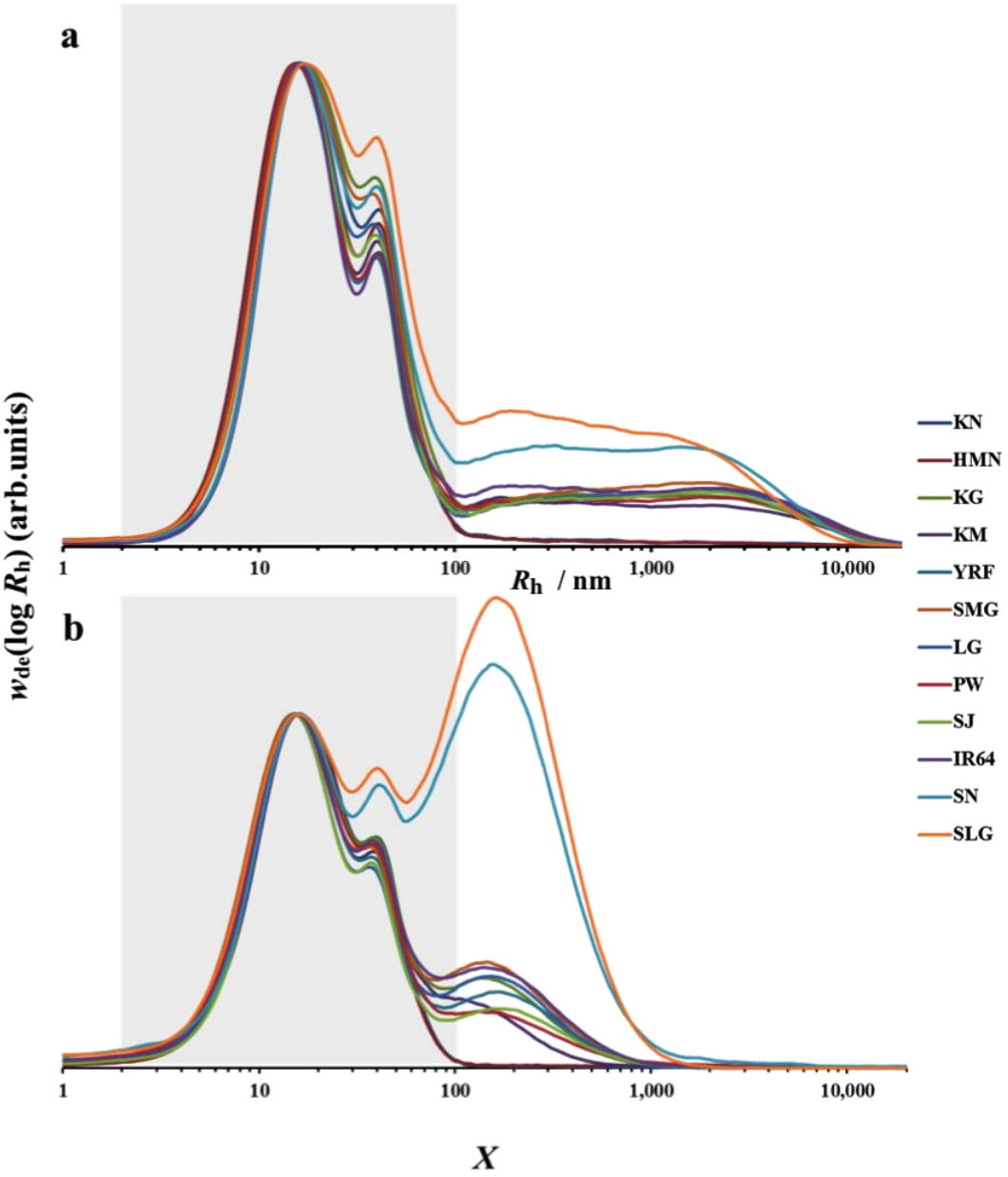

Figure 3.3 SEC weight CLDs of debranched starches. All distributions were normalized to the amylopectin peak. a) Weight CLDs for native grain starch. b) Weight CLDs for leached starch. The grey area denotes the R_h range of amylopectin in native grain and leachate, respectively.

3.3.4 The relation between leached materials, leached amylopectin molecules and stickiness

The relations between the amount and compositions of leached materials and stickiness. As shown in **Table 3.3** and the indication from the aforementioned result, rice with a higher amount of leached materials is stickier, i.e. leached material plays a significant role in

determining stickiness between grains. In **Table 3.3**, the starch and protein contents in the leached material show no significant correlations with stickiness while the total starch and protein weights in the leached material significantly correlate with stickiness. However, both the percentage and total weight of leached amylose (or amylopectin) strongly and negatively (or positively) correlate with stickiness. As displayed in **Fig. 3.1** and **Table 3.1**, waxy rices, the stickiest rices, leach amylopectin, whereas high-amylose rices, which show extremely low stickiness, leach nearly 50% of their amylose.

Relations between the molecular structure of leached amylopectin and stickiness. As displayed in **Table 3.3**, both the stickiness of cooked rice with or without hot-water washing and the stickiness loss value are positively correlated with the molecular size of leached amylopectin and the proportions of amylopectin chains with DP $\leq$ 36, and negatively correlated with the proportion of amylopectin chains with DP >36.

The effect of amylose content. As shown in **Table 3.3**, amylose content is negatively correlated with the stickiness of the freshly cooked rice, as reported elsewhere (Cameron & Wang, 2005; Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a, b). Here, it is shown for the first time that both the absolute and relative loss of stickiness by hot-water washing is negatively correlated with amylose content, meaning that rice with higher amylose content tends to reduce its stickiness to a smaller degree by hot-water washing. This can be illustrated by the negative correlation between amylose content and total amount of leached materials, i.e. rices with higher amylose content leach less during rice cooking, thereby causing less sticky texture and less stickiness loss. Also, a significant positive correlation between amylose content and the leached amylose content is seen in **Table 3.3**, where the leached amylose content can amount to 44% of the total leached starch (**Table 3.1**). Furthermore, the amylose content correlates negatively with the molecular size and proportion of short branches of leached amylopectin (**Table 3.3)**.

Table 3.3 Correlation analysis between stickiness and leaching parameters.

		Pearson Correlations																			
							Leached materials							**Molecular parameters of Leached Ap**							
		Stickiness (Freshly cooked)	Stickiness (Hot-water washed)	Stickiness Loss Value	Stickiness Loss Rate	Am content	Starch Cont	Am Cont	Protein Cont	Total solids	Starch weight	Am weight	Protein weight	$\overline{R_{h_{Ap}}}$	X_{Ap1}	X_{Ap2}	h_{Ap2}	6<X≤12	12<X≤24	24<X≤36	36<X≤100	$\bar{X}_{Ap}$
Stickiness(Freshly cooked)																					
Stickiness(Hot-water washed)		0.83**																			
Stickiness Loss Value		0.99**	0.76**																		
Stickiness Loss Rate		0.72**		0.76**																	
Amylose content		-0.93**	-0.68*	-0.94**	-0.71*																
Leached materials	Starch Content																				
	Amylose Content	-0.90**	-0.88**	-0.87**	-0.63*	0.86**															
	Protein Content																				
	Total solids	0.83**		0.85**	0.63*	-0.91**		-0.74**													
	Starch weight	0.84**		0.86**	0.65*	-0.90**		-0.73**		0.99**											
	Amylose weight	-0.86**	-0.77**	-0.85**	-0.66*	0.90**		0.93**		-0.70*	-0.68*										
	Protein weight	0.84**		0.88**	0.72**	-0.92**		-0.71**		0.86**	0.85**	-0.75**									
Molecular parameters of Leached AP	$\overline{R_{h_{AP}}}$	0.70*	0.78**	0.65*		-0.62*		-0.89**				-.075**									
	X_{Ap1}																				
	X_{Ap2}													-0.64*	0.79**						
	h_{Ap2}		-0.79**					0.81**				0.66*		-0.85**		0.82**					
	6<X≤12	0.66*	0.77**	0.61*		-0.65*		-0.83**				-0.65*		0.90**	-0.58*	-0.83**	-0.91**				
	12<X≤24	0.76**	0.88**	0.71*		-0.69*		-0.94**				-0.84**		0.89**		-0.64*	-0.95**	0.89**			
	24<X≤36	0.93**	0.78**	0.92**	0.67*	-0.90**		-0.91**		0.78**	0.78**	-0.88**	0.78**	0.76**				0.62*	0.76**		
	36<X≤100	-0.78**	-0.88**	-0.73**		0.70*		0.95**		-0.59*	-0.58*	0.81**		-0.93**		0.69*	0.94**	-0.95**	-0.98**	-0.77**	
	$\bar{X}_{AP}$																				

The content is the corresponding percentage (%) in the leached materials. The weight is the corresponding weight (mg per g rice kernel) in the leached materials, which is calculated by total solids of leached materials time the corresponding percentage. ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed);

3.4 Discussion

Starch is the main component of the rice grain. When rice is cooked, the main physical change is starch gelatinization. When starch granules swell as a result of the loss of the crystalline order and the absorption of water (Whistler, 1964), the amylose and small amylopectin molecules leach out from the granules. The leached amylose can form a three-dimensional network (Tester & Morrison, 1990). Initially, it was thought that amylose was the main leached component, and that it formed a three-dimensional network during cooling of the starch paste (Tester & Morrison, 1990). It was commonly assumed that amylose in non-waxy varieties could be separated from amylopectin by aqueous dispersion in hot water (Chinnaswamy & Bhattacharya, 1986; Kim & Willett, 2004; Roger & Colonna, 1996). Later, SEC data showed that the water-soluble fraction of non-waxy starch generally contains both amylose and smaller amylopectin molecules (Cuevas et al., 2010; Murugesan, Shibanuma & Hizukuri, 1993; Ong & Blanshard, 1995b; Ward, Gao, de Bruyn, Gilbert & Fitzgerald, 2006). In this paper we report for the first time that the molecular size of leached amylopectin is about 30 times smaller than that of grain amylopectin while the molecular size of leached amylose is about 5 times smaller than that of grain amylose, and that the CLD of leached amylopectin is similar to that of native amylopectin while that of leached amylose is over a much smaller range than that of the total amylose. Even for high-amylose rices, which leach least, the leached amylopectin content can be up to 56% of total leached starches (**Table 3.2**). In contrast to the earlier suggestion that the main leachate during gelatinization is amylose, we report that it is mainly amylopectin (at least in the varieties studied here, which cover a wide range of amylose content).

It is reported that the CLDs of amylopectin are independent of molecular size (Vilaplana & Gilbert, 2010; Wu & Gilbert, 2010). Here we also find the leached amylopectin with much smaller molecular size has a similar CLD to the native amylopectin in the region between DP 6 to 100. This study further proves that amylopectin molecules have a wide size distribution. The varieties shown in this study range in amylose content, and the region between R_h 10 – 100 nm is where amylose molecules elute (**Fig. 3.2a**). However inspection of the elution profile of the two waxy varieties shows very clearly that there are small amylopectin molecules that co-elute over the whole range of the amylose molecules, indicating that the peak spanning $R_h \sim 3 – 100$ nm consists of both amylose and small amylopectin molecules in the non-waxy varieties, and small amylopectin molecules in the waxy varieties. Comparing

the elution profiles in **Fig. 3.2**, it is clear that the amylopectin component of the leachate consists of the small amylopectin molecules. Furthermore, the leached amylopectin molecules have a smaller average chain length than the amylopectin molecules from total starch, and they have fewer chains with $X > 36$ (**Table 3.3**), which span and carry multi-clusters[35]. Together those data suggest that the smaller amylopectin molecules have fewer clusters, and fewer chains that span multiple clusters. Together those data suggest that the smaller amylopectin molecules have fewer clusters, and fewer chains that span multiple clusters.

Another noteworthy point, as shown in **Fig 3.3a**, is that native waxy starch always has a small amount of very long chains that elute in the region where amylose is usually found (up to about DP 3000), but the longest chains in the leachate of the waxy rice is about at DP 100, consistent with a previous study. It has been found that different fractions of size-separated amylopectin have similar CLDs of all but the longest chains (Laohaphatanaleart, Piyachomkwan, Sriroth & Bertoft, 2010), as also seen here, but there is a distinct difference in the CLD of the very long chains. Together with the fact that the average molecular size of the native amylopectin is about 30 times that of the leached one, we can infer that these very long amylopectin chains are the C chains which carry other short chains i.e. A- and B-chains, and span multi-clusters (more than 4), thereby contributing a significantly high molecular size, or as chains that surround and define structures such as blocklets, which are seen as the very large molecules in **Fig. 3.2a**. Even when starch is gelatinized in ordinary cooking methods (as done here), there are still water-insoluble large molecules and often some residual crystallinity. In the present work, smaller amylopectin molecules are seen to be soluble, and we speculate that these small amylopectin molecules are not linked to the main blocklet structure and are thus free to leach upon gelatinization. Consistent with this, a previous study (Cuevas, Gilbert & Fitzgerald, 2010) showed that the amount of leachate from waxy rices increased with heating across the gelatinisation endotherm, and reached a plateau at higher temperature and long heating. Thus, we can infer that, in the native starch granules, the small amylopectin molecules may entangle with large amylopectin molecules by non-covalent bonding or co-crystallize with other large amylopectin molecules, and these small ones in the leachate may be located at the edges of blocklets, and are free to leach once the crystalline structure is destroyed by heating. Therefore, the data presented here provides a

lens into the structural organisation of starch that enables a molecular explanation for the observation of small amylopectin molecules causing stickiness.

It is shown here and elsewhere (Ayabe, Kasai, Ohishi & Hatae, 2009) that stickiness increases with the total amount of leached materials and the content of leached amylopectin. Branched polymers typically exhibit shear and extensional viscosities that are unobtainable with linear polymers (Halley & George, 2009). At low shear rates, a branched polymer can exhibit a viscosity two orders of magnitude greater than that of linear polymers of the same molecular weight (McKee, Unal, Wilkes & Long, 2005). This is why a starch paste with higher amylopectin content always displays more viscous and less elastic rheological properties, while amylose molecules act as a diluent in terms of viscous properties (Lu, Sasaki, Li, Yoshihashi, Li & Kohyama, 2009). It has been shown, e.g. in the 2-dimensional data of Vilaplana, Meng, Hasjim and Gilbert (2014) that starches with higher amylose content have significant amounts of material intermediate between amylopectin and amylose in structural characteristics; these could be a component of the leached material. However, the amount of leached amylopectin is not the only reason contributing to the stickiness between cooked rice grains. It is seen here for the first time that the stickiness between cooked rice kernels is also governed by molecular size and chain length of the leached amylopectin, i.e. the more short chains, the bigger molecular size of leached amylopectin, and the greater the stickiness between cooked rice grains. For synthetic polymers, the solution rheology is strongly influenced by molecular size and branching structure. For branched polymers, chain crowding and interpenetration also constrain chain motion, thereby causing a higher viscous resistance than that of linear polymers (McKee, Unal, Wilkes & Long, 2005). Thus a higher degree of branching (as seen here in the ratio of short amylopectin ($X \leq 36$) to long amylopectin ($X > 37$) chains), together with larger molecular size of leached amylopectin, produces a higher viscous resistance, i.e. a higher stickiness.

In this study, the way that TPA measures the property termed “stickiness” (which follows the same principle as that of measuring tack in adhesion) is as follows. A single layer of cooked rice grains is placed on a baseplate. A two-cycle force/distance compression test is conducted with a probe which descends slowly (step 1: bond formation) and then is moved back (step 2: bond separation) (Friedman, Whitney & Szczesniak, 1963). Both of these clearly relate to how the human mouth would perceive the stickiness of a material to tongue and teeth during the first chew, which explains the TPA/panel data correlations (Li, Prakash, Nicholson,

Fitzgerald & Gilbert, 2016b). The quantity defined as TPA stickiness depends on a number of fundamental properties, including the bulk viscosity (Benedek, 2004).

Since TPA stickiness is the resistance offered by the cooked rice grains to detachment from the probe, the higher the stickiness value, the more force is needed to make the grains and probe come apart. As presented in section **3.3.4**, in the leachate, the stickiness increases with increasing total amount of amylopectin, the proportion of short amylopectin chains with DP ≤ 36, and amylopectin molecular size. **Fig. 3.4** shows the postulated mechanism for stickiness between cooked rice grains and the probe. There is an interface of leachate connecting the grains and the probe. Larger amylopectin molecules with higher proportions of short branches (DP ≤ 36) in the leachate can adhere to more area on the probe surface, and thus provide better bonding. On the other hand, these larger amylopectin molecules also interact with other amylopectin molecules in the leachate and in the bulk of rice kernel by H bonding, which creates viscous resistance to the detachment from the probe. The overall molecular mechanism involves H bonding between the leached small amylopectin molecule and probe, between amylopectin molecules in the leachate, and between the leached amylopectin and the bulk of the grain. An increase of the amount of amylopectin, the proportion of short amylopectin chains, and amylopectin molecular size all create a greater opportunity for bonding and molecular interaction, i.e. a higher stickiness value.

A significant effect of amylose content (arising from cultivar differences) on leaching characteristics is also seen, which could be an underlying cause of the stickiness difference between rice varieties. As shown in **Table 3.3,** the leached amylopectin content, the total amount of leached materials and the molecular size of leached amylopectin both decrease with increased amylose content. This is probably because amylose molecules are more likely to span multiple crystalline-amorphous lamellae in the grain, and to participate in the crystallization of amylopectin branches, which would restrict the starch swelling and leaching during heating.

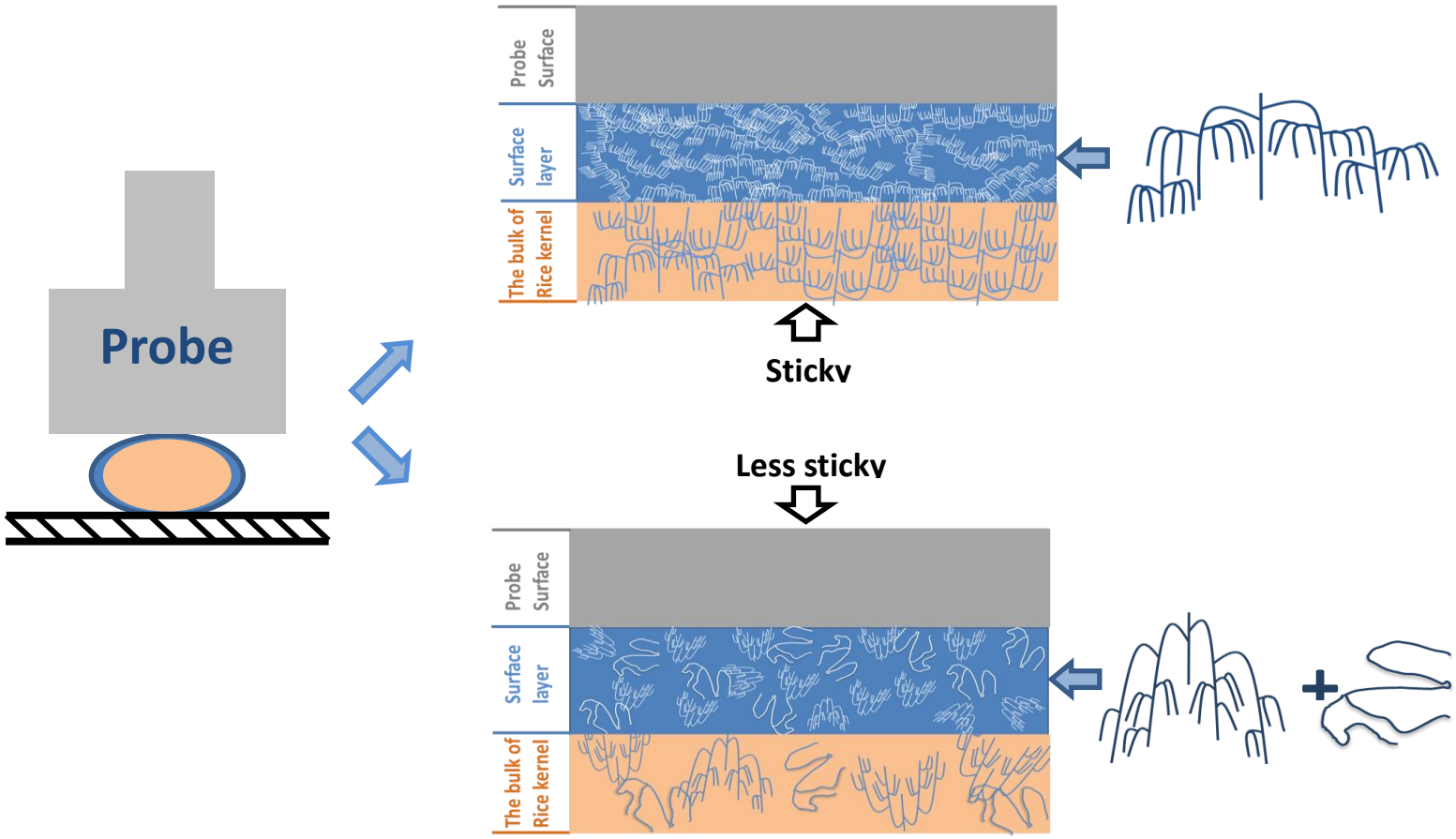

Figure 3.4 The postulated molecular mechanism for stickiness between cooked rice grains and the TPA probe. The surface layer of the sticky one has more amylopectin molecules with higher proportion of short chains with DP≤36 and bigger molecular size, while the surface layer of the less sticky one has less amylopectin molecules (diluted by amylose molecules), fewer short chains with DP≤36, and smaller molecular size.

Previous studies showed that the amount of leached amylose depends on the total amylose content (Ong & Blanshard, 1995b; Patindol, Gu & Wang, 2010), and that amylose content positively correlates with hardness and negatively correlates with stickiness (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a, b; Patindol, Gu & Wang, 2010). As shown in **Table 3.2** and **Fig. 3.4**, not only the stickiness, the leached amylose content and the total amount of leached materials, but also the molecular structural features of leached starch are also associated with amylose content. The limited swelling causes a reduction in the amount of leached materials (mainly amylopectin) and ultimately gives rise to a harder rice texture after cooking; the smaller amount of leached amylopectin, and the smaller molecular size and proportion of short branches of leached amylopectin in these cases, also contribute to a less sticky texture.

3.5 Conclusions

This study reveals that stickiness between cooked rice grains is determined by the total amount, molecular size and chain structure (CLD) of leached amylopectin. We present the first unified molecular-based mechanistic description of the causes of these important sensory properties, using the results in this study and previous findings by ourselves (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a, b) and others (Cameron & Wang, 2005; Ong & Blanshard, 1995b; Patindol, Gu & Wang, 2010). Starches with certain structural features can leach from rice kernels during cooking and attach on the surface of the cooked rice grains. The molecular size of leached amylopectin is about 30 times smaller than that of native amylopectin, while that of leached amylose is about 5 times smaller than that of grain amylose. Leached amylopectin has a similar CLD to that in the grain, while the leached amylose branches have smaller chain lengths, mainly between DP 100 – 1000. The postulated mechanism for stickiness between cooked rice grains and the probe is that an increase of the amount of amylopectin, the proportion of short amylopectin chains, and amylopectin molecular size in the leachate all create a greater opportunity for bonding and molecular interaction, causing more force to be needed to make the grains and probe come apart, i.e. a higher stickiness value.

An underlying origin of the stickiness differences between rice cultivars is the amylose content in the whole grain starch. With increasing amylose content, the total amount of leached materials, the amylopectin content in the leachate, and the molecular size and the proportion of short branches of leached amylopectin, all decrease, leading to a lower

stickiness. However, amylose content is not the sole determinant. In some cases, amylose content is similar but the hardness (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a) and/or stickiness (Ayabe, Kasai, Ohishi & Hatae, 2009) still vary significantly. This is because of the effects of other structural features. One such is amylose chain-length distributions. Our previous finding points out that high-amylose rice tends to have higher proportions of short amylose chains (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a). Whether this is a characteristic of all high-amylose rices could provide insight into their functional differences. Another determining structural feature is the interaction between amylose and amylopectin molecules (the location of amylose) in native starch granules. The location of amylose in native starch granules is not completely understood, but it is often thought that amylose molecules are present in an amorphous conformation (Lopez-Rubio, Flanagan, Shrestha, Gidley & Gilbert, 2008; Morrison, Law & Snape, 1993); further, there are suggestions that amylose is spread among amylopectin crystallites (Jane, Xu, Radosavljevic & Seib, 1992; Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a), and may co-crystallize with amylopectin chains.

By quantifying the components and the molecular structure of leached starch, rice breeders could choose lines which optimize the texture of cultivars. For example, a cultivar which leaches more amylopectin with more short amylopectin chains and bigger molecular size would be sticky after cooking, which could be desirable for sushi. On the other hand, a cultivar which leaches more amylose should be less sticky but have a harder texture. This molecular structural mechanism provides a new tool for rice breeders to select cultivars with desirable palatability.

Chapter 4 Instrumental measurement of cooked rice texture by dynamic rheological testing and its relation to the fine structure of rice starch

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Chapter abstract: Increasing demands for better instrumental methods to evaluate cooked rice texture, which is less costly and time-consuming but more accurate, is driving innovations in rice texture research. This study characterized cooked rice texture by descriptive sensory analysis and two instrumental methods (texture profile analysis (TPA) and dynamic rheological test) using a set of 18 varieties of rice with a wide range in amylose content (0-30%). Panellists' results indicated that hardness and stickiness were the two most discriminating attributes among 13 tested textural attributes. Consistency coefficient (K^*) and loss tangent (tan δ) from dynamic frequency sweep were used to compare with hardness and stickiness tested by TPA and sensory panellists, showing that K^* representing hardness and tan δ representing stickiness are both statistically and mechanism-meaningful. The instrumental method is rationalized in terms of starch structural differences between rices: a higher proportion of both amylose and long amylopectin branches with DP 70–100 causes a more elastic and less viscous texture, which is readily understood in terms of polymer dynamics in solution.

4.1 Introduction

Rice is a major staple food world-wide. In recent years, consumer preferences have shifted towards better-quality rice, particularly towards varieties with good eating quality. Each country, and often region, prefers rice with a particular suite of quality traits. The textural attributes of cooked milled rice are of prime importance to its eating quality(Calingacion et al., 2014). Descriptive sensory analysis is an objective tool used to characterize textural traits of foods (Meilgaard, Carr & Civille, 2006). The technique has been used extensively for determining the effect of different growing and/or processing conditions on sensory properties of rice (Champagne et al., 2010; Lyon, Champagne, Vinyard & Windham, 2000; Lyon et al., 1999). However, the cost associated with training and maintaining a sensory panel has prompted many researchers to evaluate less costly and less time-consuming instrumental approaches.

Most rice is consumed in the form of grains, rather than after processing to flour, which raises challenges in collecting rheological data that is relevant to the sensory experience of eating rice. As such, texture analysers, where individual grains can be placed on a plate, are currently the most commonly used instruments to measure the texture of cooked rice kernels(Ayabe, Kasai, Ohishi & Hatae, 2009). This method has been employed with some success and, in some cases, provides data that relate closely to sensory evaluation data (Prakash, Ravi, Sathish, Shyamala, Shwetha & Rangarao, 2005; Sesmat & Meullenet, 2001). However, its limitations restrain further applications. The texture analyser is used to obtain the force-displacement curve by a double-compression test of typically two rice kernels, which is less reliable and accurate than a test performed on bulk samples(Juliano et al., 1984). The poor repeatability of this method is also reported when conducted on freshly cooked rice, due to the rapid retrogradation of rice starch with rice decreasing temperature, which, consequently, resulted in more replicates and complex sample preparation needed to obtain statistically meaningful data (Meullenet, Gross, Marks & Daniels, 1998). Furthermore, the range of geometries available for texture analysers has also meant that standard fixtures and procedures are not always used, which makes it difficult to compare studies. While not always practiced, measurements should always be reported as stress rather than force to allow comparisons to be made between geometries and methodologies (Stokes, Boehm & Baier, 2013). TPA mimics the first bite of a food sample (Stokes, Boehm & Baier, 2013), corresponding to Phase II in **Table S4.1**. There has been extensive work using rheometers to

measure properties of food materials in relation to food microstructure and sensory texture (Chen & Stokes, 2012; Foegeding et al., 2011). Compared to conventional texture-profile analyser (TPA) measurements, rheological studies have the benefits of a well-defined geometry and deformation process, related to fundamental mechanical parameters (such as stress, strain, strain rate, storage and loss moduli, etc.) for quantitative descriptions of food materials. However, while rheological properties have been extensively investigated to relate to the texture/mouthfeel of liquid and semi-fluid foods, there are unanswered questions relating to their use for semi-solid or solid foods (Foegeding et al., 2011), e.g. cooked white rice.

Starch is the major component of rice grains, and starch structure is considered to be the most important factor affecting the cooking quality of rice, e.g. gelatinization temperature (Cuevas et al., 2010), starch swelling (Hasjim, Li & Dhital, 2012), starch leaching (Patindol, Gu & Wang, 2010), correspondingly, determines the texture of cooked rice. Amylose content has since the mid-1980s been considered to be the most important determinant of the eating quality of rice (Bhattacharya & Juliano, 1985). In the mid-1990s, it was proposed that the texture of cooked rice is also related to the fine structure of amylopectin (Reddy, Ali & Bhattacharya, 1993). In previous work, we found that the fine structure of amylose, both molecular size and chain-length distribution, are also significant determinants of the hardness of cooked rice (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a).

In this study, properties of a diverse set of rices with a wide range of amylose contents were evaluated by a trained panel and two instruments. A novel instrumental method, dynamic rheology with vane geometry, was developed to compare with the conventional TPA method and with textural perceptions of a sensory panel. The fine structure of amylopectin and amylose was measured to identify the structural origins of the textural differences between rice samples.

4.2 Materials and methods

4.2.1 Materials

18 milled rice grain samples were chosen from a collection of rice varieties with known phenotypes and genotypes for quality traits (**Table 4.1**). After harvesting, all rice samples were dehulled in a dehusker (Otake, Aichi, Japan), polished to yield rice with the same

whiteness value in a commercial mill (FASCO, VIC, Australia), and then stored in self-sealing plastic bags in a refrigerator before subsequent analyses.

Protease from *Streptomyces griseus* (type XIV), and LiBr (ReagentPlus) were purchased from Sigma-Aldrich Pty. Ltd. (Castle Hill, Australia). Isoamylase (from *Pseudomonas sp.*) and a D-glucose (glucose oxidase/peroxidase; GODOP) assay kit were purchased from Megazyme International, Ltd. (Wicklow, Ireland). 8-Aminopyrene-1,3,6,-trisulfonate (APTS), included in the Carbohydrate Labelling and Analysis Kit, was purchased from Beckman Coulter (Brea, USA). A series of pullulan standards with peak molecular weights ranging from 342 to 2.35 × 10^6 were from Polymer Standards Service (PSS) GmbH (Mainz, Germany). Dimethyl sulfoxide (DMSO, GR grade for analysis) was from Merck Co. Inc. (Kilsyth, Australia). All other chemicals were reagent-grade and used as received.

4.2.2 Rice cooking

Rice (600 g, 14% moisture content) was rinsed with distilled water three times. As shown in **Table 4.1**, distilled water was then added to the rice to give rice-to-water weight ratios for three different cooking types based on amylose content (1:1.3, 0%; 1:1.6, 10-25%; 1:1.8, 25-30%). The high ratio for high-amylose rices is often used; such rices do not become sticky after cooking even with this high ratio. The cooking process was conducted using the pre-set cooking setting of a rice cooker (Kambrook Rice Express, VIC, Australia), followed by a 10 min holding period at the warming setting. The top 1 cm layer of cooked rice and rice adhering to the sides of rice cooker were not used. Cooked rice for sampling was taken directly from the middle of each cooker, transferred to a pre-warmed (120 ℃) glass bowl, and mixed thoroughly while minimizing kernel breakage. The glass bowl was then kept in a 50 ℃ water bath for sensory evaluation.

4.2.3 Sensory evaluation protocol

10 panellists trained in the principles and concepts of descriptive sensory analysis (Meilgaard, Carr & Civille, 2006) participated in the study. The sensory lexicon included 13 sensory attributes that described rice texture at different phases of eating, beginning with the feel of the rice when it is first placed in the mouth and ending with mouthfeel characteristics after the rice swallowed (**Table S4.1** in the supplementary data). Each sample was presented to the panellists twice, following a randomized design in which each session consisted of four samples, a standard, and a blind control (Sunrice® long grain, a commercial cultivar). The

standard, which was the warm-up sample presented at the beginning of each session, was used to calibrate the panel. After the warm-up sample, coded test samples were presented to panellists individually at 20 min intervals immediately after cooking, holding, and portioning into serving cups. Evaluations were conducted at individual test stations. Spring water was used to cleanse the mouth between samples.

4.2.4 TPA

A 1 g subsample of cooked rice grains was weighed and placed as a single layer of grains on a flat glass dish. Then TPA measurements were conducted using the method described previously (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a).

4.2.5 Dynamic viscoelasticity measurement

Dynamic viscoelasticity measurements were carried out in a stress-controlled rheometer AR G2 (TA instruments, USA) with a controlled temperature Peltier element set at 37 ℃. The geometry used was specially designed for small sample volumes. A four-bladed vane geometry with a diameter of 15 mm and a length of 15 mm, and a cup with a diameter of 40 mm were used (TA instruments, USA). After cooking, 25 g of cooked rice grains were immediately loaded into the cup and gently packed to remove air. The vane was then set down to a distance of 4 mm from the bottom of the cup and was completely immersed in the rice bulk. No mineral oil was added to the top of the cooked rice kernels to avoid mixing with the food bolus. After the vane temperature decreased to 37 ℃, the rice bolus was allowed to rest for 5 min before the following tests were implemented.

Two dynamic tests were performed: (a) An oscillatory stress sweep test from 0.1 to 1000 Pa, at a constant frequency of 10 rad/s and 37 ℃, was made to set the upper limit of the linear viscoelastic region (LVR). (b) Frequency sweep over a range of 0.1-100 rad/s at 37 ℃ was performed at the oscillatory stress of 2 Pa, which is within LVR for all rice samples. Viscoelastic parameters, storage or elastic modulus (G', Pa), loss or viscous modulus (G'', Pa), and loss tangent ($\tan \delta = G''/G'$) as a function of angular frequency (ω, rad/s) were measured.

Silicon oil is often used to minimize water loss for a suspension in a rheometer, but that cannot be used here since it would mix with rice kernels and affect the measurements. However, the effect of water loss on these rheological measurements should be negligible, because: 1) the total time for the frequency sweep test is 25 min, which is not long enough to lose much water; 2) the vane was set to a distance of 4 mm from the bottom of the cup and

was completely immersed in the rice bulk. Hence the top layer of rice will reduce water loss in the middle and bottom layers of rice, which are those in contact with the vane.

4.2.6 Size- exclusion chromatography

All starch samples were extracted and dissolved in a DMSO solution with 0.5% (w/w) LiBr (DMSO/LiBr), following a method described elsewhere (Syahariza, Li & Hasjim, 2010). The extracted starch was then debranched using isoamylase following a method described elsewhere (Wang, Hasjim, Wu, Henry & Gilbert, 2014; Wu, Li & Gilbert, 2014). To obtain the size-exclusion chromatography (SEC) distributions of debranched starch, GRAM 100 and GRAM 1000 columns (PSS) were used at a flow rate of 0.6 mL/min. The SEC weight distribution, $w(\log X)$, obtained from the DRI signal was plotted against X (DP), with X being determined using the Mark-Houwink relationship in terms of the hydrodynamic volume, viz. the SEC separation parameter $V_h = {}^2/_5\, KM^{1+\alpha}/N_A$, with molecular weight $M = 162.2(X–1) + 18.0$ (162.2 is the molecular weight of the anhydroglucose monomeric unit and 18.0 is that of the additional water in the end groups) and N_A the Avogadro constant; the Mark-Houwink parameters K and α for linear starch chains in the eluent of DMSO/LiBr at 80 ℃ are 1.5×10^{-4} dL g^{-1} and 0.743, respectively (Cave, Seabrook, Gidley & Gilbert, 2009). The full branched distributions are reported as the SEC weight distribution as functions of the hydrodynamic radius Rh, $w(\log R_h)$, with $V_h = {}^3/_4\ \pi R_h^3$.

The amylose content of all rices was determined from the SEC weight distributions of debranched starch following the procedure described by Syahariza, Sar, Hasjim, Tizzotti and Gilbert (2013). This method has been shown to be more accurate than the iodine colorimetric method (Fitzgerald et al., 2009; Vilaplana, Hasjim & Gilbert, 2012).

4.2.7 Fluorophore-assisted carbohydrate electrophoresis (FACE)

The debranched starch, prepared in the same way as that for SEC analysis, was labelled using APTS following a procedure described by Wu, Li and Gilbert (2014), and then separated with a carbohydrate separation buffer (Beckman-Coulter) in an N-CHO coated capillary using an applied voltage of 30 kV (current ~14 mA) at 25 ℃. The number chain-length distribution (CLD), $N_{de}(X)$, of debranched amylopectin was characterized using a PA-800 Plus FACE system (Beckman-Coulter, USA), coupled with a solid-state laser-induced fluorescence (LIF) detector with an argon-ion laser as the excitation source.

4.2.8 Fitting amylopectin number CLDs with a biosynthesis model

The number CLDs of amylopectin from FACE were used with an amylopectin biosynthesis based model to obtain information on the starch biosynthetic enzymes. The underlying theory is described elsewhere (Wu & Gilbert, 2010; Wu, Morell & Gilbert, 2013). In summary, the amylopectin CLD is attributed to a number of enzyme sets, with each set comprising various isoforms of starch synthases (SS), starch branching enzymes (SBE) and debranching enzymes (DBE), with a given set predominantly but not exclusively contributing to a particular range of the CLD. The resulting parameters are the activity ratio of SBE to SS (denoted β_1, β_2 and β_3) of each enzyme set and the relative contributions of enzyme sets 2 and 3 relative to that of enzyme set 1 (denoted $h_{2/1}$ and $h_{3/1}$). The role of phosphorylase in forming enzyme complexes between different enzymes and isoforms of these (Tetlow et al., 2008; Tetlow, Morell & Emes, 2004; Tetlow et al., 2004) contributes to the action of each enzyme set. One important use of this model is that the parameters obtained by fitting (the β and *h* values) accurately express the whole CLD (including quite subtle features (Wu, Witt & Gilbert, 2013)) in a small number of physically meaningful parameters. This is a major improvement over representing a CLD by empirical parameterizations such as fractions in arbitrarily chosen ranges of DP. Of special significant for the present work is that these parameters are ideal for finding statistically significant correlations between structure and properties. These parameters can be obtained from CLD data (FACE, HPAEC or SEC) using publicly available code (Wu & Gilbert, 2013).

4.2.9 Statistical analysis

The sensory data were first analysed using the general linear model for ANOVA for each textural attribute in Minitab® 16 (Minitab Inc., USA). A principal components analysis (PCA) from SIMCA software (Umetrics, Sweden) was used to extract the first two dimensions in all textural attributes data that explained the greatest amount of variation. For each instrumental and structural measurement, duplicated analyses were performed for each sample. All data were reported as mean and standard deviation (SD) using ANOVA with Tukey's pairwise comparisons. Significant differences of the mean values were determined at $p < 0.05$. One-way ANOVA and Pearson rank correlation methods were carried out using SPSS V. 16.0 software (SPSS Inc., USA). The means of duplicated measurements were used for the correlation analysis.

4.3 Results and discussion

4.3.1 Rice texture

Human perception of cooked rice texture

The sensory scores of textural attributes of all cooked rice samples are significantly different (**Table S4.2** in the Supplementary data), except for springiness and moisture absorption. Springiness and moisture absorption data are therefore omitted from the PCA analysis. As shown in **Fig. 4.1**, the score plot shows that the two principal components (PC), PC1 and PC2, explain 73.7% and 12.4% of variations of all textural attributes, respectively. The loading plot shows the clustering of the varieties based on all textural profiles which of variations are explained by PC1 (73.7%) and PC2 (12.4%). As shown in **Fig. 4.1a**, hardness, residual loose particles and roughness (defined as "hard group" attributes) are loaded at about –0.3, indicating these three attributes are strongly and positively correlated with each other. Stickiness to lips, stickiness between grains, cohesiveness, cohesiveness of mass, uniformity of bite, initial starchy coating, and toothpack (defined as "sticky group' attributes), loaded at about 0.3, are also significantly and positively correlated. Correspondingly, these two groups of attributes are negatively correlated with each other. Further, the score plot of first two components (**Fig. 4.1b**) points out the separation of rice samples into three main groups according to hierarchical clustering analysis (**Fig. S4.1** in the Supplementary data): waxy rices (HMN and SN); high-amylose rices (SLG and SN); and the remaining low and intermediate-amylose rices, indicating a clear effect of amylose content on rice classification. Combining **Fig. 4.1a** and **Fig. 4.1b**, high-amylose rices (SLG and SN) contribute significantly on attributes of the "hard group", since high-amylose rices are always hard, rough, and with a significant number of loose particles tending to remain in the mouth after swallowing; waxy rices (KN and HMN) contribute significantly to attributes of the "sticky group", because waxy rices are always sticky, uniform between bites, and easily stick on the surface of teeth. This is consistent with other reported results about the relationship between textural attributes (Lyon et al., 1999; Park, Kim & Kim, 2001), and also further confirmed the wide-accepted conclusion that high-amylose rice tends to have hard and less sticky texture, and vice versa. On the other hand, it also is seen that the presence of extreme samples (waxy and high-amylose rices) make the classification of low-amylose rices difficult; hardness and stickiness are two most discriminable attributes when rices with a wide range of amylose content are applied.

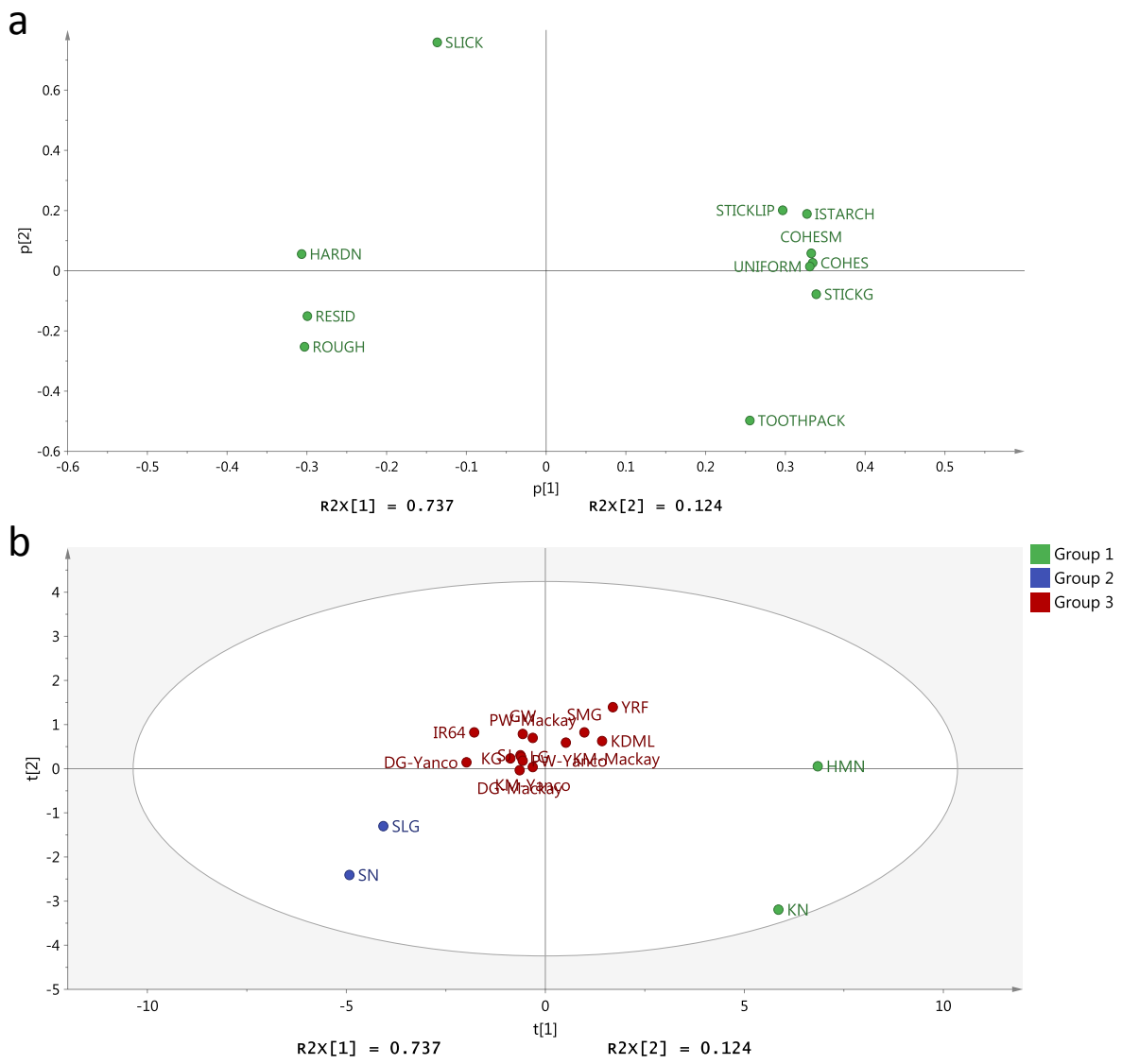


Figure 4.1 PCA of textural sensory data of all rice samples by panellists. (a) Loading scatter plot: textural attributes of cooked rice on first two principal components. (b) Score scatter plot: rice varieties on first two principal components. (ISTARCH: Initial starchy coating; SLICK: Slickness; ROUGH: Roughness; STICKLIP: Stickiness to lips; STICKG: Stickiness between grains: COHES: Cohesiveness; HARDN: Hardness; UNIFORM: Uniformity of bite; COHESM: Cohesiveness of Mass; RESID: Residual loose particles; TOOTHPACK: Toothpack). Abbreviations of samples given in Table 4.1.

Dynamic rheology of cooked rice grains

As shown in **Fig. 4.2**, all rice samples have similar profiles of mechanical spectra. Generally, the values of the storage modulus (G') of all samples are ~5 times higher than those of the loss modulus (G''), exhibiting a solid-like characteristics for all cooked rices over the range of frequencies measured. G' increases with an increase in the frequency, which is commonly a behaviour seen with suspensions; this can be explained because the cooled rice kernels adhere to each other after cooking, causing the rice bolus to be more like a gel, rather than discrete particles. Both G'' and tan δ show slight minima, decreasing in the low frequency range (0.1-1 rad s–1) and increasing at high frequency (1-100 rad s–1); this may be due to the thixotropic behaviour. There is nearly no effect of holding time on the slope of G' : a preliminary time sweep test showed that all rice samples had a similar increase (about 10%) of G' in the first 30 min, the time required for the whole frequency sweep, so the effect of holding time on the slope of G' is negligible. Further, a significant effect of amylose content on the mechanical spectra can be observed (**Fig. 4.2**), showing that rices with higher amylose content tend to have higher G' and lower G'' and tan δ, and vice versa. On the other hand, specific differences between samples are also apparent. As shown in **Fig. 4.2a**, KN and HMN show higher slopes of the G' curve than do other rices (especially high-amylose rices e.g. SLG and SN). Lu, Sasaki, Li, Yoshihashi, Li and Kohyama (2009) investigated the effect of amylose content and rice type on dynamic viscoelasticity of a composite rice starch gel, and reported that the behaviour of low-amylose rice is more frequency-dependent than high-amylose rice. This also indicates that, regardless of rice morphology (rice kernel or rice gel), amylose content significantly affects the frequency dependence of G'.

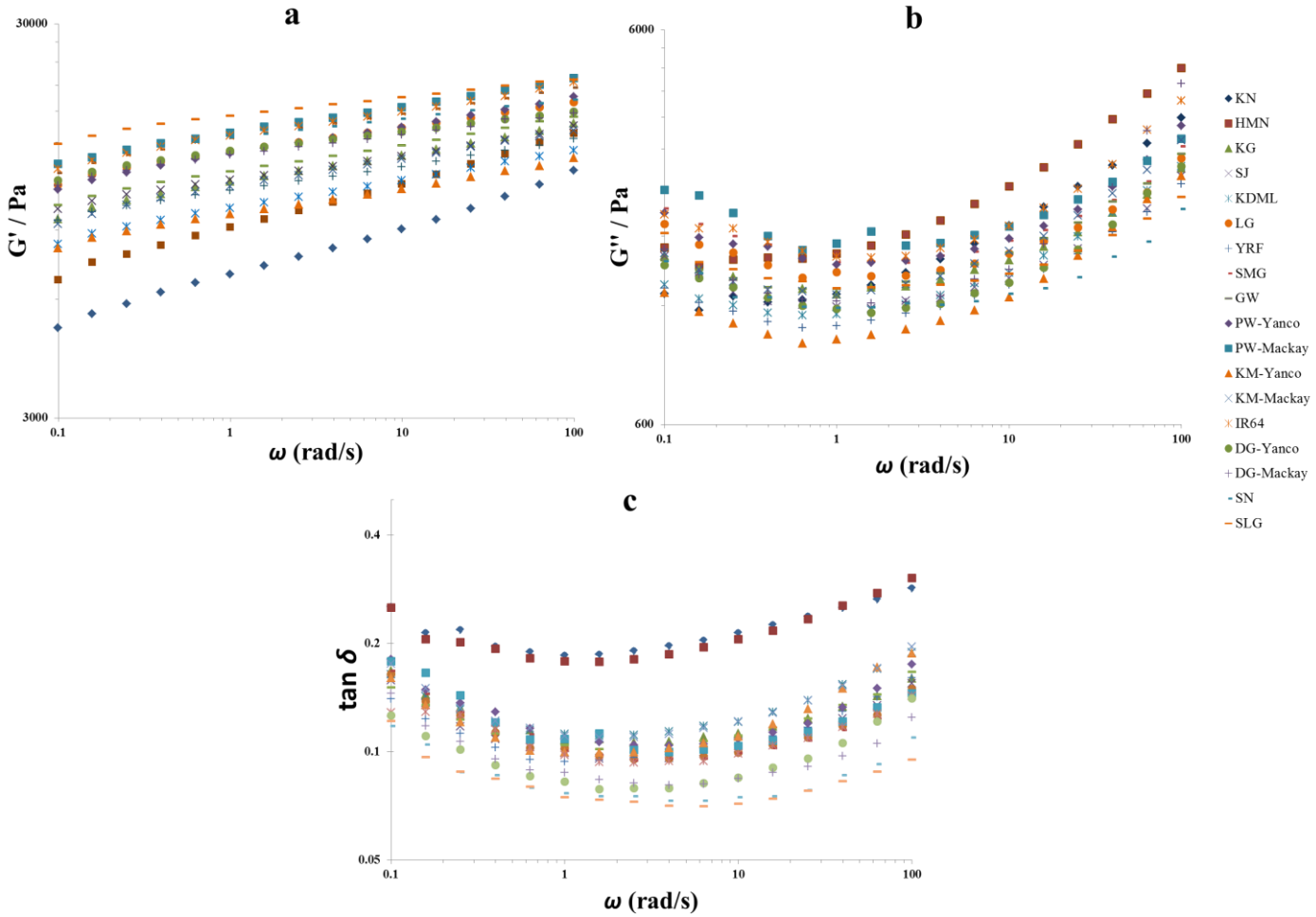


Figure 4.2 Dynamic viscoelasticity as a function of frequency for all cooked rice samples: a. dynamic storage moduli (G'); b. dynamic loss moduli (G''); and c. loss tangent (tan δ).

To further parameterize the mechanical spectra of all samples, the viscosity power law model is applied to the small deformation oscillation (Holdsworth, 1971):

$$\eta^* = K^* \dot{\gamma}^{n^*-1}$$

where η^* is the dynamic viscosity, K^* the consistency constant, $\dot{\gamma}$ the frequency, and n^* the power law index or flow behaviour index for dynamic viscosity.

As indicated from **Fig. 4.2**, $G' \gg G''$, so G' significantly dominates the complex modulus; thus the values of K^* and n^* together effectively express the elastic properties of the cooked rice samples. The mechanical loss factor tan δ represents the viscous properties of rice samples. K^*, n^*, and tan δ were selected to compare between samples in **Table 4.1**. As shown in **Table 4.1**, the values of n^* of all cooked rice samples $\ll 1$ (0.072–0.137), indicating a significant shear thinning behaviour, i.e. viscosity decreases with frequency or increasing shear rate. Between rice samples, high-amylose rice tends to have smaller value of n^* than waxy rices, indicating a relatively stronger shear-thinning behaviour. In contrast, high-amylose rices have much bigger K^* than waxy or low-amylose rices, indicating high-amylose rices tend to have a higher viscosity at a specific frequency. However, these trends are not simply and linearly correlated with amylose content: for example, YRF has similar amylose content to SMG (**Table 4.2**), but the K^* of SMG is significantly higher than that of YRF (**Table 4.1**). Although both $K^*$and n^* display elastic characteristics of cooked rice, n^* shows less significant variations between samples in comparison to K^*. The values of n^* are significantly different from other rices only for waxy rices (KN and HMN), and thus K^* is preferred to represent the elasticity of cooked rices. Since the tan δ profiles of all rice samples are similar, and are nearly parallel curves (**Fig. 4.2**), the tan δ at 10 rad s–1 was picked to parameterize the differences of viscous characteristics between samples. 10 rad s–1 corresponds to a frequency of 1.59 s^{-1}, which is close to human masticatory frequency (Jalabert-Malbos, Mishellany-Dutour, Woda & Peyron, 2007). As presented in **Table 4.1**, tan δ shows the opposite trend to K^*: waxy rices tend to have higher tan δ values while high-amylose rices have smaller ones.

Table 4.1 Rice variety and corresponding cooking, dynamic rheological, and TPA results of all rice samples.[a]

Rice varieties	Abbreviation code	Country of Origin	Cooking	Dynamic Rheology			TPA	
			Water: rice	K^*	n^*	tan δ @10 rad/s	Hardness	Stickiness
Khao Niao	KN	Thailand	1.3:1	1460 ± 273 [a]	0.135 ± 0.004 [b]	0.214 ± 0.016 [e]	2470 ± 270 [a,b]	280 ± 106 [g]
Hom Mali Niaw	HMN	Australia	1.3:1	1951 ± 113 [a-c]	0.137 ± 0.006 [b]	0.205 ± 0.007 [e]	2200 ± 266 [a]	307 ± 62 [g]
Kangaroo	KG	Australia	1.6:1	2346 ± 108 [b-g]	0.099 ± 0.013 [a]	0.112 ± 0.002 [c,d]	3255 ± 510 [c-e]	173 ± 45 [e,f]
Sunrice Jasmine	SJ	Australia	1.6:1	2249 ± 153 [b-e]	0.076 ± 0.009 [a]	0.102 ± 0.004 [c]	3396 ± 264 [e]	120 ± 19 [c,d]
Khao Dawk Mali 105	KDML	Australia	1.6:1	1986 ± 91 [a-c]	0.095 ± 0.004 [a]	0.121 ± 0.001 [d]	2755 ± 245 [a-d]	143 ± 15 [d-f]
Langi	LG	Australia	1.6:1	2749 ± 158 [d-h]	0.090 ± 0.007 [a]	0.100 ± 0.003 [b,c]	3290 ± 348 [d,e]	116 ± 17 [c,e]
YRF209	YRF	Australia	1.6:1	2126 ± 303 [a-d]	0.080 ± 0.007 [a]	0.104 ± 0.002 [c]	2680 ± 338 [a-c]	127 ± 28 [c,d]
Sunrice Medium grain	SMG	Australia	1.6:1	2995 ± 289 [g,h]	0.094 ± 0.009 [a]	0.099 ± 0.002 [b,c]	3523 ± 225 [e-g]	184 ± 37 [f]
Golden way	GW	Australia	1.6:1	2427 ± 127 [b-g]	0.090 ± 0.011 [a]	0.111 ± 0.003 [c,d]	3115 ± 240 [c-e]	138 ± 14 [d-f]
Pandan Wangi (Yanco)	PW-Yanco	Australia	1.6:1	2951 ± 189 [f-h]	0.078 ± 0.025 [a]	0.108 ± 0.002 [c]	3093 ± 136 [c-e]	133 ± 16 [d-f]
Pandan Wangi(Mackay)	PW-Mackay	Australia	1.6:1	2703 ± 102 [d-h]	0.093 ± 0.006 [a]	0.103 ± 0.003 [c,d]	2988 ± 314 [b-e]	137 ± 30 [d-f]
Kyeema (Yanco)	KM-Yanco	Australia	1.6:1	2289 ± 110 [b-f]	0.101 ± 0.014 [a]	0.110 ± 0.004 [d]	2725 ± 475 [a-d]	135 ± 25 [d-f]
Kyeema (Mackay)	KM-Mackay	Australia	1.6:1	1906 ± 21 [a,b]	0.091 ± 0.003 [a]	0.121 ± 0.002 [c,d]	2772 ± 290 [a-d]	147 ± 32 [d-f]
IR64	IR64	Australia	1.6:1	2966 ± 392 [f-h]	0.086 ± 0.010 [a]	0.099 ± 0.001 [b,c]	3442 ± 447 [e,f]	101 ± 34 [b,d]
Doongara (Yanco)	DG-Yanco	Australia	1.8:1	2614 ± 102 [c-h]	0.080 ± 0.004 [a]	0.085 ± 0.001 [a,b]	2780 ± 300 [a-d]	48 ± 12 [a,b]
Doongara (Mackay)	DG-Mackay	Australia	1.8:1	2683 ± 290 [d-h]	0.079 ± 0.004 [a]	0.084 ± 0.004 [a,b]	3097 ± 245 [c-e]	50 ± 22 [a,c]
Swarna	SN	Australia	1.8:1	2858 ± 69 [e-h]	0.072 ± 0.002 [a]	0.075 ± 0.001 [a]	4010 ± 340 [f,g]	15 ± 5 [a]
Sunrice Long Grain	SLG	Thailand	1.8:1	3288 ± 521 [h]	0.077 ± 0.009 [a]	0.072 ± 0.001 [a]	4054 ± 530 [g]	33 ± 7 [a]

[a] Mean ± SD calculated from duplicate measurements. Values with different letters in the same column are significantly different with $p < 0.05$.

Table 4.2 Starch molecular parameters extracted from SEC and model fitting parameters for all rice samples.[a]

Rice varieties	$h_{2/1}$	$h_{3/1}$	β_1	β_2	β_3	Amylose Content	$100<X\leq500$	$500<X\leq5000$	$5000<X\leq20000$	X_{Am}	h_{Am}
KN	0.084 ±0.001 [a]	0.0053 ±0.0001 [a]	0.091 ±0.000 [a]	0.052 ±0.000 [a-d]	0.065 ±0.001 [e]	-	-	-	-	-	-
HMN	0.99 ±0.006 [a-c]	0.0055 ±0.0003 [a,b]	0.089 ±0.001 [a]	0.056 ±0.001 [a-d]	0.073 ±0.001 [b-e]	-	-	-	-	-	-
KG	0.121 ±0.001 [b,c]	0.0096 ±0.0001 [d-f]	0.093 ±0.000 [a]	0.048 ±0.000 [a,b]	0.052 ±0.000 [b-d]	18.78 ±0.51 % [a]	6.12 ±0.48 % [a]	9.84 ±0.11 % [a-c]	2.55 ±0.07 % [a,b]	807 ±78 [a-f]	0.098 ±0.005 [a-c]
SJ	0.086 ±0.005 [a,b]	0.0070 ±0.0002 [a-e]	0.135 ±0.005 [e]	0.061 ±0.000 [d,e]	0.062 ±0.003 [d,e]	20.31 ±0.57 % [a-d]	6.56 ±1.24 % [a,b]	10.81 ±0.31 % [b-e]	2.78 ±0.19 % [b-d]	999 ±214 [c-f]	0.106 ±0.004 [b-e]
KDML	0.079 ±0.019 [a]	0.0062 ±0.0016 [a-c]	0.101 ±0.011 [a-d]	0.056 ±0.001 [a-e]	0.048 ±0.001 [b,c]	19.11 ±0.48 % [a,b]	6.80 ±0.24 % [a,b]	9.29 ±0.15 % [a]	2.80 ±0.05 % [b-d]	722 ±43 [a-c]	0.092 ±0.005 [a]
LG	0.078 ±0.013 [a]	0.0066 ±0.0007 [a-c]	0.102 ±0.019 [a-d]	0.060 ±0.010 [c-e]	0.044 ±0.010 [a,b]	20.46 ±0.18 % [b-d]	6.00 ±0.16 % [a]	11.04 ±0.13 % [c-e]	3.10 ±0.10 % [b-f]	1098 ±43 [f]	0.103 ±0.004 [a-d]
YRF	0.092 ±0.016 [a-c]	0.0061 ±0.0011 [a,b]	0.107 ±0.006 [a-e]	0.079 ±0.001 [f]	0.064 ±0.000 [d,e]	21.20 ±0.40 % [c,d]	6.58 ±0.11 % [a,b]	11.14 ±0.49 % [c-f]	3.21 ±0.18 % [c-f]	994 ±0 [c-f]	0.112 ±0.000 [d,e]
SMG	0.088 ±0.009 [a,b]	0.0100 ±0.0007 [f]	0.095 ±0.001 [a,b]	0.046 ±0.001 [a]	0.034 ±0.000 [a]	20.79 ±0.33 % [c,d]	6.06 ±0.20 % [a]	11.47 ±0.09 % [e,f]	2.96 ±0.34 % [b-e]	1059 ±41 [e,f]	0.115 ±0.001 [d-f]
GW	0.081 ±0.010 [a]	0.0067 ±0.0009 [a-d]	0.101 ±0.001 [a-d]	0.061 ±0.003 [c-e]	0.043 ±0.000 [a,b]	22.87 ±0.19 % [e]	7.04 ±0.14 % [a,b]	12.35 ±0.16 % [f,g]	3.19 ±0.09 % [c-f]	959 ±24 [b-f]	0.128 ±0.004 [f]
PW-Yanco	0.093 ±0.004 [a-c]	0.0061 ±0.0002 [a,b]	0.108 ±0.004 [a-e]	0.063 ±0.001 [d,e]	0.063 ±0.002 [d,e]	21.82 ±0.68 % [d,e]	6.72 ±0.29 % [a,b]	11.38 ±0.63 % [d-f]	3.45 ±0.13 % [e,f]	1051 ±81 [d-f]	0.111 ±0.003 [c-e]
PW-Mackay	0.094 ±0.000 [a-c]	0.0069 ±0.0004 [a-e]	0.098 ±0.000 [a-d]	0.055 ±0.001 [a-e]	0.052 ±0.001 [b-d]	20.27 ±0.36 % [a-c]	6.93 ±0.36 % [a,b]	10.15 ±0.28 % [a-d]	2.95 ±0.07 % [b-e]	830 ±111 [a-f]	0.102 ±0.001 [a-d]
KM-Yanco	0.087 ±0.005 [a,b]	0.0070 ±0.0002 [a-e]	0.135 ±0.005 [e]	0.062 ±0.000 [d,e]	0.063 ±0.003 [d,e]	20.86 ±0.33 % [c,d]	6.38 ±0.10 % [a]	10.89 ±0.42 % [b-e]	3.22 ±0.09 % [d-f]	977 ±49 [c-f]	0.102 ±0.006 [a-d]
KM-Mackay	0.105 ±0.012 [a-c]	0.0079 ±0.0015 [a-f]	0.127 ±0.003 [d,e]	0.059 ±0.003 [b-e]	0.059 ±0.000 [c-e]	19.98 ±0.37 % [a-c]	7.38 ±0.16 % [a-c]	9.62 ±0.23 % [a,b]	2.66 ±0.02 % [b,c]	677 ±71 [a,b]	0.093 ±0.003 [a,b]
IR64	0.123 ±0.004 [e]	0.0098 ±0.0007 [e,f]	0.108 ±0.001 [a-e]	0.058 ±0.003 [b-e]	0.036 ±0.000 [a]	23.13 ±0.05 % [e]	8.06 ±0.03 % [b-d]	11.58 ±0.20 % [e,f]	3.27 ±0.11 % [d-f]	759 ±27 [a-d]	0.117 ±0.002 [e,f]
DG-Yanco	0.098 ±0.008 [a-c]	0.0083 ±0.0003 [b-f]	0.123 ±0.009 [b-e]	0.066 ±0.001 [e]	0.062 ±0.002 [d,e]	26.64 ±0.19 % [f]	8.69 ±0.04 % [c,d]	14.51 ±0.53 % [h]	3.31 ±0.05 % [d-f]	950 ±12 [b-f]	0.155 ±0.000 [g]
DG-Mackay	0.110 ±0.008 [a-c]	0.0095 ±0.0006 [d-f]	0.119 ±0.003 [a-e]	0.060 ±0.001 [c-e]	0.061 ±0.001 [d,e]	26.55 ±0.44 % [f]	9.58 ±0.58 % [d,e]	13.17 ±0.50 % [g]	3.58 ±0.05 % [f]	766 ±55 [a-e]	0.143 ±0.004 [g]
SN	0.102 ±0.001 [a-c]	0.0091 ±0.0007 [c-f]	0.125 ±0.016 [c-e]	0.059 ±0.002 [b-e]	0.054 ±0.001 [b-e]	30.34 ±0.16 % [g]	11.11 ±0.23 % [e]	16.17 ±0.02 % [i]	2.92 ±0.03 % [b-e]	605 ±20 [a-f]	0.200 ±0.002 [h]
SLG	0.103 ±0.001 [a-c]	0.0106 ±0.0001 [f]	0.098 ±0.006 [a-c]	0.050 ±0.001 [a-c]	0.044 ±0.001 [a,b]	30.81 ±0.37 % [g]	13.18 ±0.08 % [f]	15.58 ±0.15 % [h,i]	2.00 ±0.23 % [a]	593 ±33 [a]	0.227 ±0.002 [i]

[a] Mean ± SD is calculated from duplicate measurements. Values with different letters in the same column are significantly different with $p < 0.05$

TPA of cooked rice grains

As presented in **Table 4.1**, apparently, rice with higher hardness tends to have smaller stickiness, indicating a negative correlation between hardness and stickiness. This is consistent with the panellists' results, and the results in our previous work (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a). It is noteworthy that rices samples in this study are cooked with a range of water/rice ratios. Greater amounts of water decrease the rice hardness (Bett-Garber, Champagne, Ingram & McClung, 2007), and it is seen here that the hardness difference between rice samples decreases with increased water/rice ratio.

4.3.2 Starch fine structure

The populations at DPs smaller than 100 are normally assigned as amylopectin chains, while that of DPs above 100 are amylose chains, although there are almost certainly a small proportion of extra-long amylopectin chains with lengths similar to shorter amylose chains in this region (Hanashiro et al., 2008). While FACE gives highly accurate amylopectin CLDs (Gilbert, Witt & Hasjim, 2013), the technique cannot be used for large chains (currently going up to X ~180 (Wu, Li & Gilbert, 2014)), and thus the chain lengths of amylopectin and amylose branches are analysed by FACE and SEC, respectively.

Fig. 4.3a shows the typical number CLDs of amylopectin branches from FACE. All samples show the well-known features as previously reported for rice starch (Wu, Morell & Gilbert, 2013). Four peaks and/or shoulders can be observed for all rice samples. The first peak is the global maximum at DP ~12, followed by a small bump at approximately DP ~21. These two features, covering DP ~6–32, are short amylopectin chains confined to one crystalline lamella (single-lamella chains). A local minimum is observed at DP ~33, separating single- and trans-lamella branches, the latter spanning two or more crystalline lamellae. The populations with maximum at DP ~44 are trans-lamella branches that span through one crystalline lamella and the adjacent amorphous lamella, while the other feature with a local maximum at DP ~75 is long amylopectin branches spanning at least two adjacent crystalline lamellae and the amorphous lamellae in between (Wu, Morell & Gilbert, 2013). As in previous studies (Wu & Gilbert, 2010), the amylopectin number CLDs are fitted with the amylopectin biosynthesis model (**Fig. S4.2** in the Supplementary data) to quantify the differences between samples. By model fitting, three β values (β_1, β_2 and β_3), each representing the relative activity of SBE to SS within each enzyme set, and another set of parameters $h_{2/1}$ and $h_{3/1}$ reflecting the relative contributions of enzyme sets 2 and 3 to that of enzyme set 1 are obtained. As shown in **Table**

4.2, all parameters are significantly different over these samples. High and intermediate-amylose rices (DG, SN and SLG) tend to have higher values of $h_{2/1}$ and $h_{3/1}$, and smaller values of β_2 and β_3, showing that enzyme sets 2 and 3 have a lower SBE activity, and/or a higher SS activity, consequently causing a higher proportion of long amylopectin branches. These three varieties are known to carry haplotype 1 of SSIIa, (G/G/GC), which is a more active form of the enzyme (Cuevas et al., 2010), therefore suggesting that SS activity explains the differences in values. This method of obtaining statistically useful information by fitting to the biosynthesis-based model is very much to be preferred over the older method of dividing the CLD into arbitrarily chosen DP ranges and using the proportions of each; this older method is empirical, and different results can be obtained if different ranges are chosen.

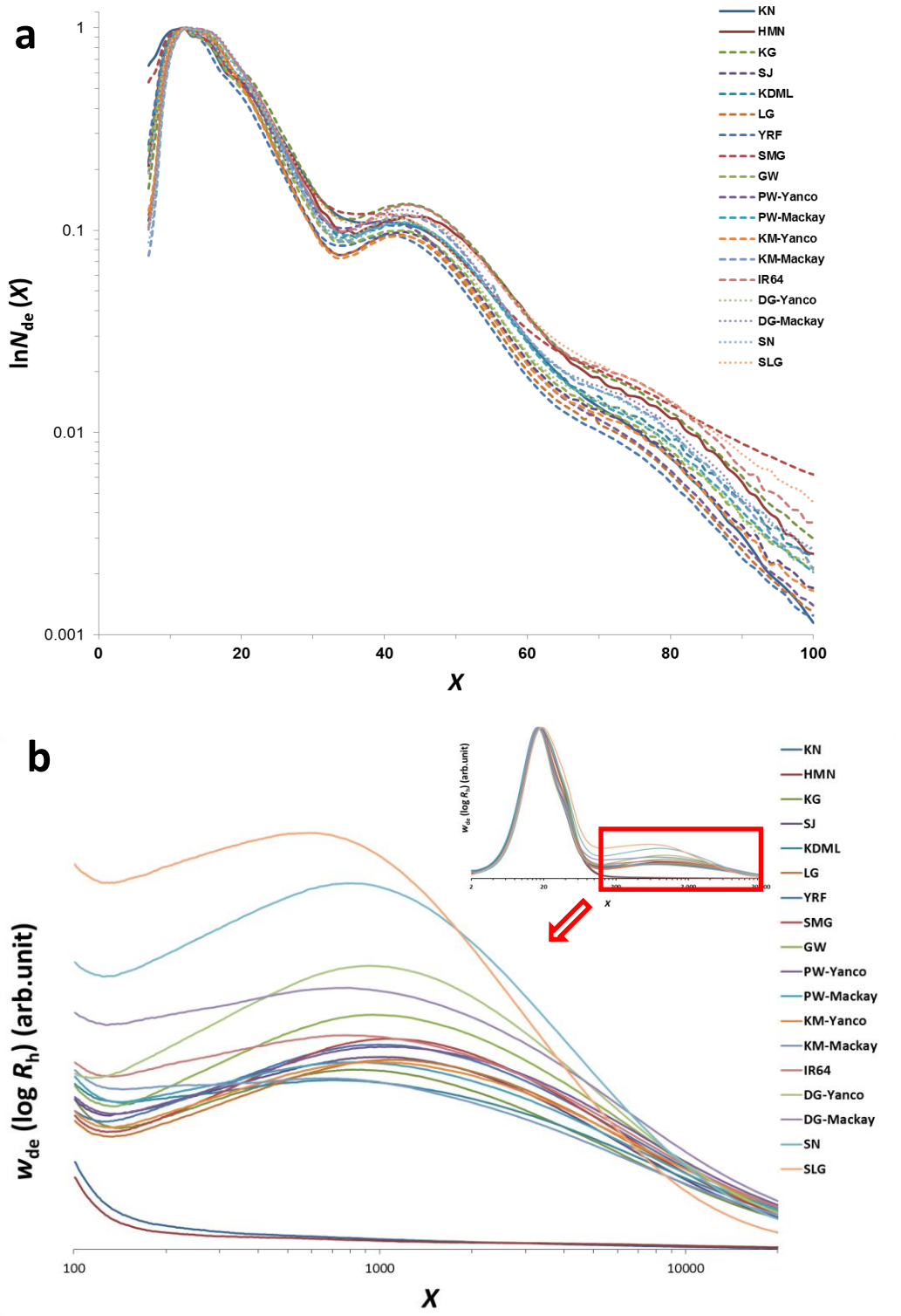


Figure 4.3 (a) FACE number CLDs of debranched amylopectin branches; (b) debranched amylose branches (the small insert at the top-right corner is SEC weight CLDs of the whole range of debranched starch branches); All distributions are normalized to the highest amylopectin peak.

Fig. 4.3b presents typical SEC weight distributions of debranched amylose branches for all rice samples. As there is yet no quantitative model for amylose biosynthesis, and moreover these data suffer from some distortion due to SEC band broadening, a set of empirical parameters is used to compare the structural differences of amylose between samples. These are the DP at the maximum of amylose peak, donated X_{Am}, and the height ratio of maximum of amylose peak to that of maximum of amylopectin peak, h_{Am}. The X range of amylose is further subdivided into 3 different fractions, $100 \leq X < 500$, $500 \leq X < 5000$, and $5000 \leq X < 20000$. The percentage of the area under the curve (AUC) for each fraction is also calculated as previously (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a). Amylose is synthesized by the *Waxy* (*Wx*) gene, which encodes granule-bound starch synthase. Different haplotypes of the *Wx* gene are defined by single nucleotide polymorphisms (SNPs) at exon 1 and 6, which affect the amount of amylose accumulated (Chen, Bergman, Pinson & Fjellstrom, 2008). Waxy varieties contain a duplication in exon 2 of the *Wx* that completely disables transcription, so waxy varieties produce no amylose (Wanchana, Toojinda, Tragoonrung & Vanavichit, 2003). As shown in **Table 4.2**, the varieties used in the present paper have previously been genotyped at the *Wx* locus(Calingacion et al., 2014). All rice varieties containing amylose can be divided into 3 categories, consistent with the *Wx* haplotype, defined by functional SNPs at exons 1 and 6 of the *Wx*. These are low-amylose rice varieties which all contain T at exon 1 (KG, SJ, KDML, LG, YRF, SMG, GW, PW, and KM; amylose content ~0–20%); two varieties, IR64 and DG, with haplotype G-C of the *Wx* gene with intermediate amylose (amylose content~25%); and high amylose rice, with *Wx* haplotype G-A (SLG and SN; amylose content ~30%). There are significant structural differences between these three categories of rice. For rice varieties with intermediate and high amylose content, and with G at exon 1 of the *Wx* gene, X_{Am} tends to be smaller than for those with low amylose and T at exon 1 of the *Wx*. This could indicate that rice with a functional allele of *Wx* contains more short branches, which is also consistent with our previous finding (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a).

4.3.3 Comparison between rheological data and TPA in measuring hardness and stickiness of cooked rice grains

Since TPA measures hardness and stickiness while dynamic rheological measurements determines elastic and viscous characteristics, it is reasonable to compare both instrumental methods and/or replace TPA with the dynamic rheological test. The panellists' perception of

hardness and stickiness (stickiness to lips, STL) were also selected to assess if both instrumental measurements reflect human perceptions. As discussed previously (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a), starch fine structure is a significant determinant of the texture of cooked rice, and thus the correlation between starch structure and rice texture were analysed to explain the textural and rheological differences between samples.

Table 4.3 summarizes the coefficients from Pearson's rank correlation test between starch structure and rice texture measured instrumentally (rheology and TPA) and by panellists; the correlation between two instrumental measurements and panellist's perception of texture is also included. As indicated from **Fig. 4.1**, waxy rices (KN and HMN) and high-amylose rices (SN and SLG) are extreme samples contributing significantly to the variations of rice textural attributes, especially to hardness and stickiness, so correlations with the exclusion of extreme rices (waxy and high-amylose rices) are also presented in **Table 4.3** to demonstrate the differences in the correlations with a narrow range of amylose content.

The structural basis for texture (hardness and stickiness) and rheological property

Among all these structural parameters, $100 \leq X < 500$, $500 \leq X < 5000$, $5000 \leq X < 20000$, and h_{Am} are all directly related to amylose content. For all rice samples, all of these parameters except $5000 \leq X < 20000$, along with amylose content, show positive correlations with hardness, and negative correlations with stickiness. For samples excluding waxy and high-amylose rices, $500 \leq X < 5000$ still positively correlates with hardness tested by panellists. Correspondingly, the parameters of β_1, β_2, β_3, $h_{2/1}$, and $h_{3/1}$ represent the chain-length and content of amylopectin chains. For all rice samples, $h_{3/1}$ is positively correlated with hardness, and β_3 shows negative correlation with hardness, indicating that the proportion and chain-length of long trans-lamella amylopectin chains with DP $70 \leq X < 100$ also positively correlate with hardness. This confirmed our previous results (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a).

K^* displays similar correlation with starch structure to hardness, i.e. positively correlated with amylose parameters (amylose content, $100 \leq X < 500$, $500 \leq X < 5000$, and h_{Am}) and the content of long amylopectin chains ($h_{3/1}$) while negatively correlated with the chain-length of long amylopectin chains (β_3). Correspondingly, tan δ shows analogous correlations with structural parameters to stickiness, i.e. negatively correlated with amylose parameters (amylose content, $100 \leq X < 500$, $500 \leq X < 5000$, and h_{Am}) and the content of long amylopectin chains ($h_{3/1}$).

Table 4.3 Correlation coefficients between starch structure and rice texture tested by instrumental (Rheology and TPA) and panellists.[a]

Structural and textural parameters	All rice samples						Rice samples without waxy and high-amylose rices					
	Rheology		TPA		Panellists		Rheology		TPA		Panellists	
	K^*	tan δ[b]	Hardness	Stickiness	Hardness	STL[c]	K^*	tan δ	Hardness	Stickiness	Hardness	STL
$h_{2/1}$	0.282	-0.238	0.259	-0.242	0.426	-0.443	0.163	-0.278	0.193	-0.189	0.4	-0.504
$h_{3/1}$	0.666**	-0.639**	0.731**	-0.573*	0.675**	-0.727**	0.388	-0.43	0.51	-0.084	0.405	-0.516
β_1	0.026	-0.441	0.117	-0.534*	0.295	-0.35	-0.336	-0.119	-0.312	-0.451	0.022	-0.229
β_2	-0.131	-0.204	-0.287	-0.286	0.074	-0.036	-0.274	-0.181	-0.556*	-0.481	0.016	-0.11
β_3	-0.584*	0.469*	-0.633**	0.3	-0.468	0.313	-0.443	-0.049	-0.612*	-0.353	-0.252	-0.037
Am Content	0.742**	-0.975**	0.732**	-0.959**	0.824**	-0.837**	0.397	-0.790**	-0.051	-0.862**	0.598*	-0.857**
$100<X\leq500$	0.518*	-0.823**	0.618*	-0.871**	0.663**	-0.926**	0.145	-0.641*	-0.163	-0.839**	0.427	-0.894**
$500<X\leq5000$	0.621*	-0.902**	0.618*	-0.877**	0.776**	-0.881**	0.468	-0.797**	0.055	-0.746**	0.634*	-0.711**
$5000<X\leq20000$	-0.023	0.108	-0.462	-0.003	-0.032	0.123	0.532	-0.531	-0.081	-0.665**	0.467	-0.590*
X_{Am}	0.005	0.217	-0.225	0.356	-0.163	0.504*	0.391	-0.18	0.252	0.089	0.145	0.292
h_{Am}	0.617*	-0.876**	0.700**	-0.845**	0.730**	-0.891**	0.413	-0.801**	0.027	-0.756**	0.603*	-0.762**
K^*	1						1					
tan δ	-0.749**	1					-0.547*	1				
Hardness(TPA)	0.784**	-0.723**	1				0.633*	-0.294	1			
Stickiness	-0.664**	0.916**	-0.676**	1			-0.181	0.717**	0.185	1		
Hardness(Panellists)	0.823**	-0.798**	0.799**	-0.805**	1		0.612*	-0.588*	0.563*	-0.488	1	
STICKLIP	-0.685**	0.752**	-0.730**	0.903**	-0.818**	1	-0.357	0.724**	-0.077	0.870**	-0.603*	1

[a] * Correlations are significant at $p < 0.05$; ** Correlations are significant at $p < 0.01$;
[b] tan δ at frequency of 10 rad/s;
[c] STL: stickiness to lips

During rice heating, starch granules swell as a result of the loss of the crystalline order starch and the absorption of water, while the amylose inside the granules leaches out simultaneously . The swelling behaviour of rice starch is primarily controlled by amylopectin, and amylose acts as both a diluent and inhibitor of swelling (Tester & Morrison, 1990). Therefore, high-amylose rice is more resistant to swelling, leading to more elastic (higher K^*) and less viscous (lower tan δ) characteristics. The long amylopectin chains also tend to restrict starch swelling (Ong & Blanshard, 1995a; Radhika Reddy, Zakiuddin Ali & Bhattacharya, 1993). Ong and Blanshard (1995) proposed that the long amylopectin chains may crystallize with an amylose molecule, which might extend through several adjacent 'clusters', thereby contributing to double helices in several crystallites and which could result in a lower degree of swelling, a reduction in the leaching of material, ultimately giving rise to a harder (more elastic) and less sticky (less viscous) texture. These reports are consistent with our present results. Furthermore, after cooking, the initial stage of gelation of starch is dominated by the gelation of the solubilized amylose, which plays a key role in the gelation and initial retrogradation of starch (Miles, Morris, Orford & Ring, 1985); this is another possible explanation for why high-amylose rices are more elastic and less viscous. This indicates that starch leaching and the structure of leached starch should be investigated in the future work.

Rheological measurements as an alternative to TPA

For all rice samples (**Table 4.3**), K^* is positively correlated with hardness measurements from TPA and panellists (p<0.01), while tan δ shows positive correlations with stickiness from TPA and stickiness to lips by panellists (p<0.01). For rice samples without waxy and high-amylose rices, K^* is still positively correlated with hardness by TPA and panellists (p<0.05) while tan δ still shows positive correlations with stickiness by TPA and stickiness to lips by panellists (p<0.01).

The correlations between rheological parameters (K^* and tan δ) and texture (hardness and stickiness) are mechanistically meaningful. K^*, known as the consistency index, represents the viscosity at a shear rate of unity (Holdsworth, 1971). In this study, $G' \gg G''$ **(Fig. 4.2)**, so G' dominates the complex modulus, and thus K^* effectively represents the elastic characteristics of cooked rice samples. Higher K^* represents higher elasticity, i.e. a harder texture. tan δ, the mechanical loss factor, is a measure of the energy dissipated during a

loading cycle relative the energy stored elastically in the material (Mark, 1996). It directly reflects viscous characteristics, corresponding to the stickiness of cooked rices.

Although TPA equipment is less expensive, the rheological method overcomes the limitations of conventional TPA measurement, e.g. good reproducibility, easy operability, etc. Further, it is carried out on the bulk of cooked white rice, instead of a couple of rice kernels, which is closer to human perceptions.

4.4 Conclusions

The current study shows that sensory descriptive analysis and two instrumental methods for evaluating the hardness and stickiness of cooked rice are significantly correlated. Specifically, K^* positively correlates with hardness tested by TPA and panellists while tan δ shows positive correlations with stickiness by TPA and stickiness to lips by panellists. On the other hand, K^* represents the elasticity of cooked rice while tan δ represents viscous characteristics, indicating it is also mechanistically meaningful as well as preferable to use dynamic rheological testing as an alternative to TPA. Further, the present novel instrumental method overcomes certain limitations of conventional TPA measurement: good reproducibility and easy operability. Additionally, it is carried out on bulk of cooked white rice, instead of a couple of rice kernels, which is closer to human perceptions.

The differences in fine structures of amylopectin and amylose can be seen to be causally controlling the textural differences between rice samples. Amylose content and the proportion of long amylopectin branches ($70 \leq X < 100$) are positively correlated with K^* tested by dynamic rheology and hardness tested by TPA and panellists, indicating that rices with higher content of both amylose and long amylopectin branches might be resistant to swelling during cooking, correspondingly, causing a more elastic and less viscous texture. This can be ascribed to entanglement of these longer chains slowing down the swelling process, equivalent to well-known mechanical effects with synthetic polymers. This also suggests that rice swelling and starch leaching in relation to rice texture should be further investigated in future work.

4.5 Supplementary data

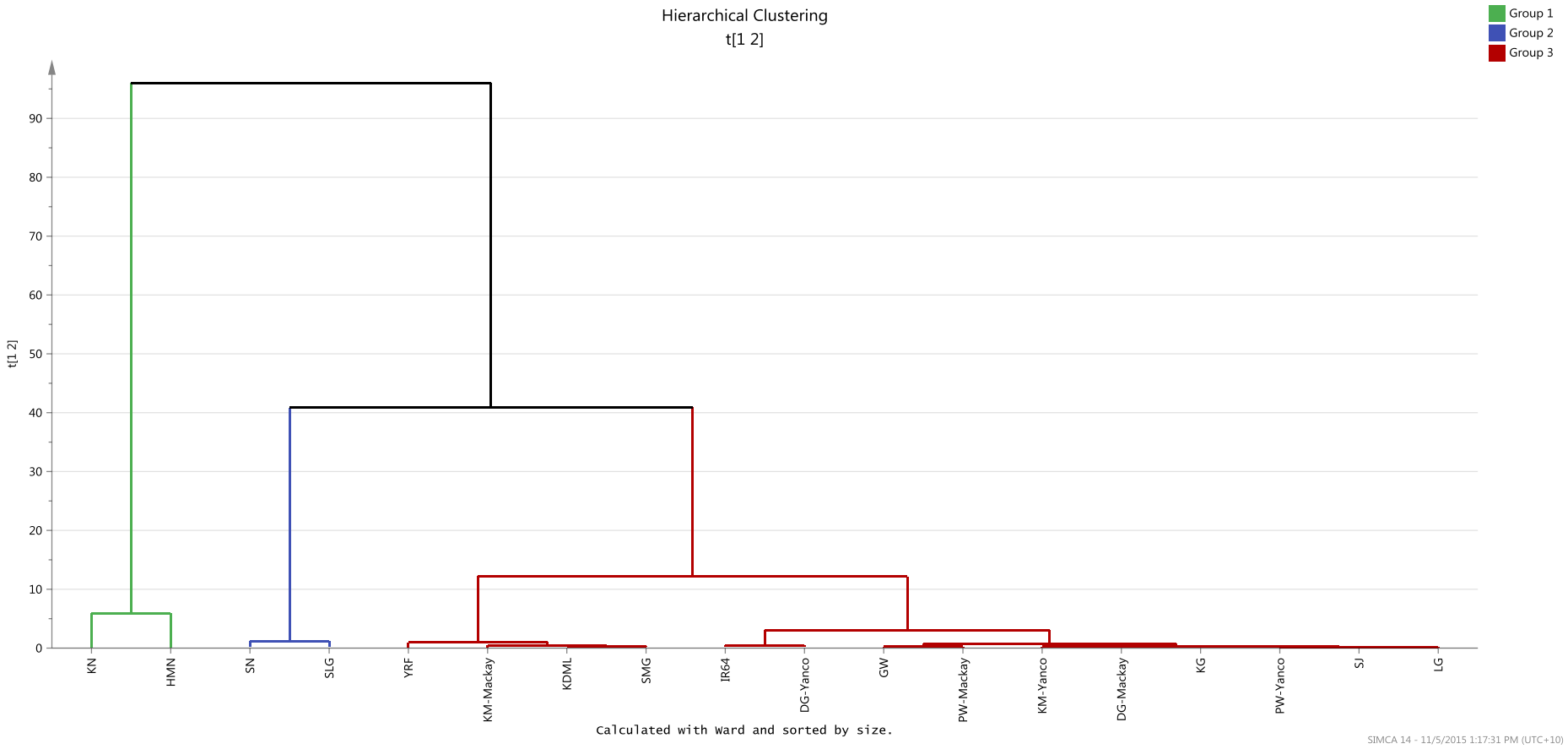
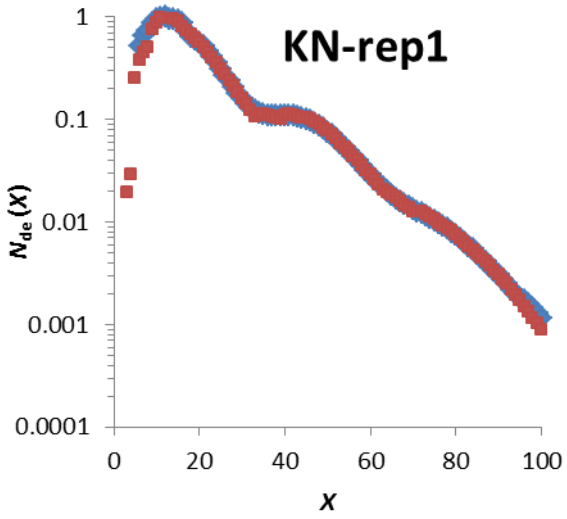


Figure S 4.1 Hierarchical clustering analysis for rices classification.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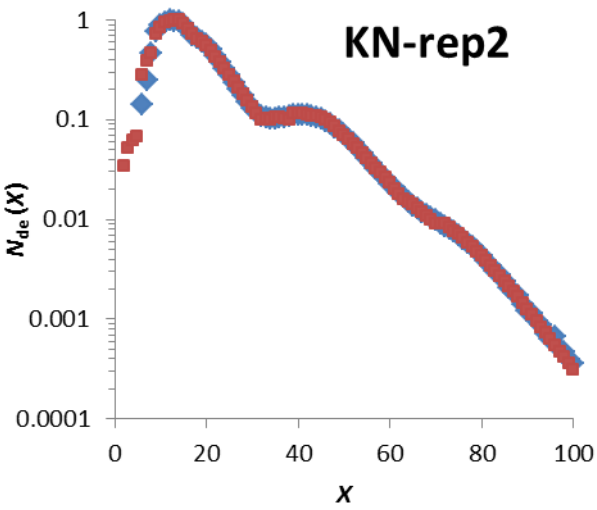
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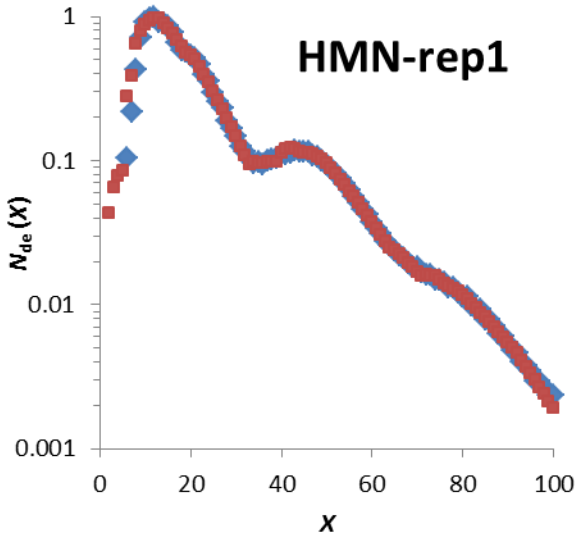
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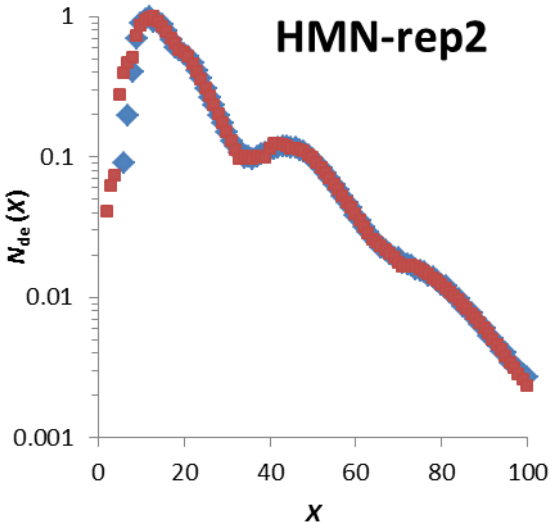
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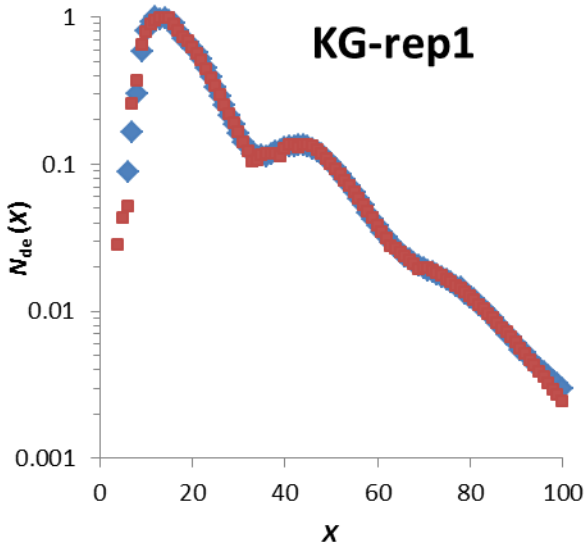
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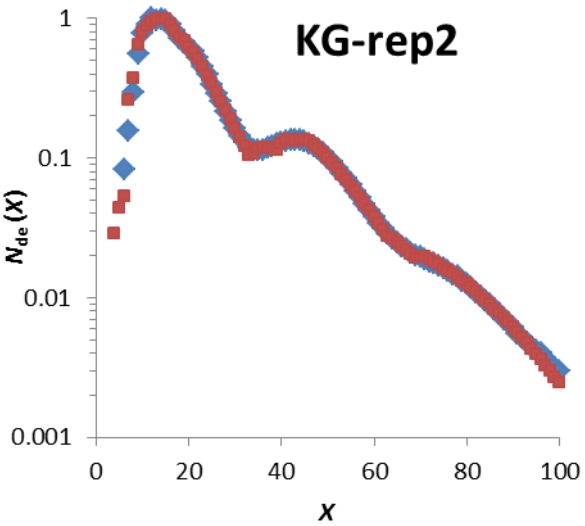
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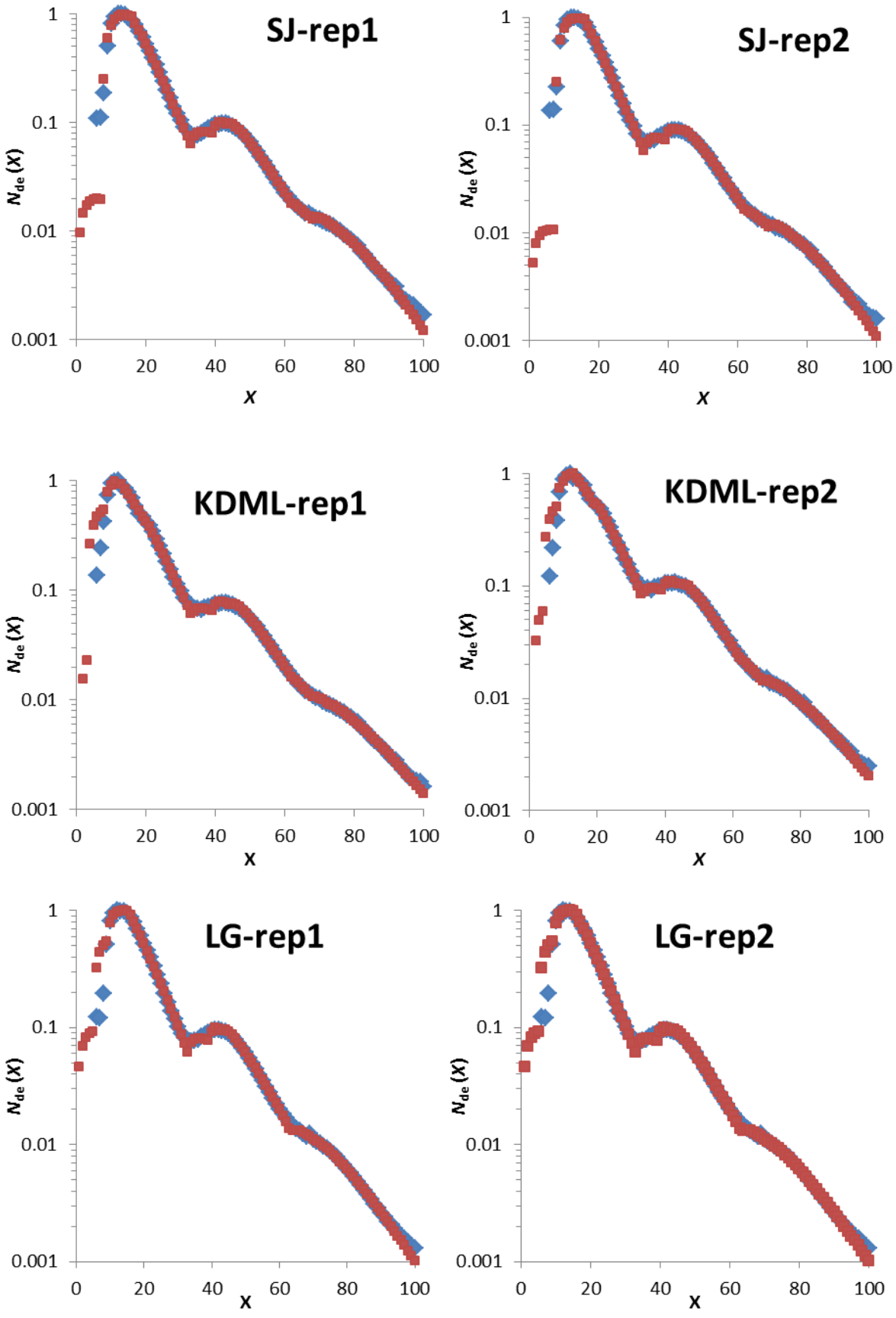
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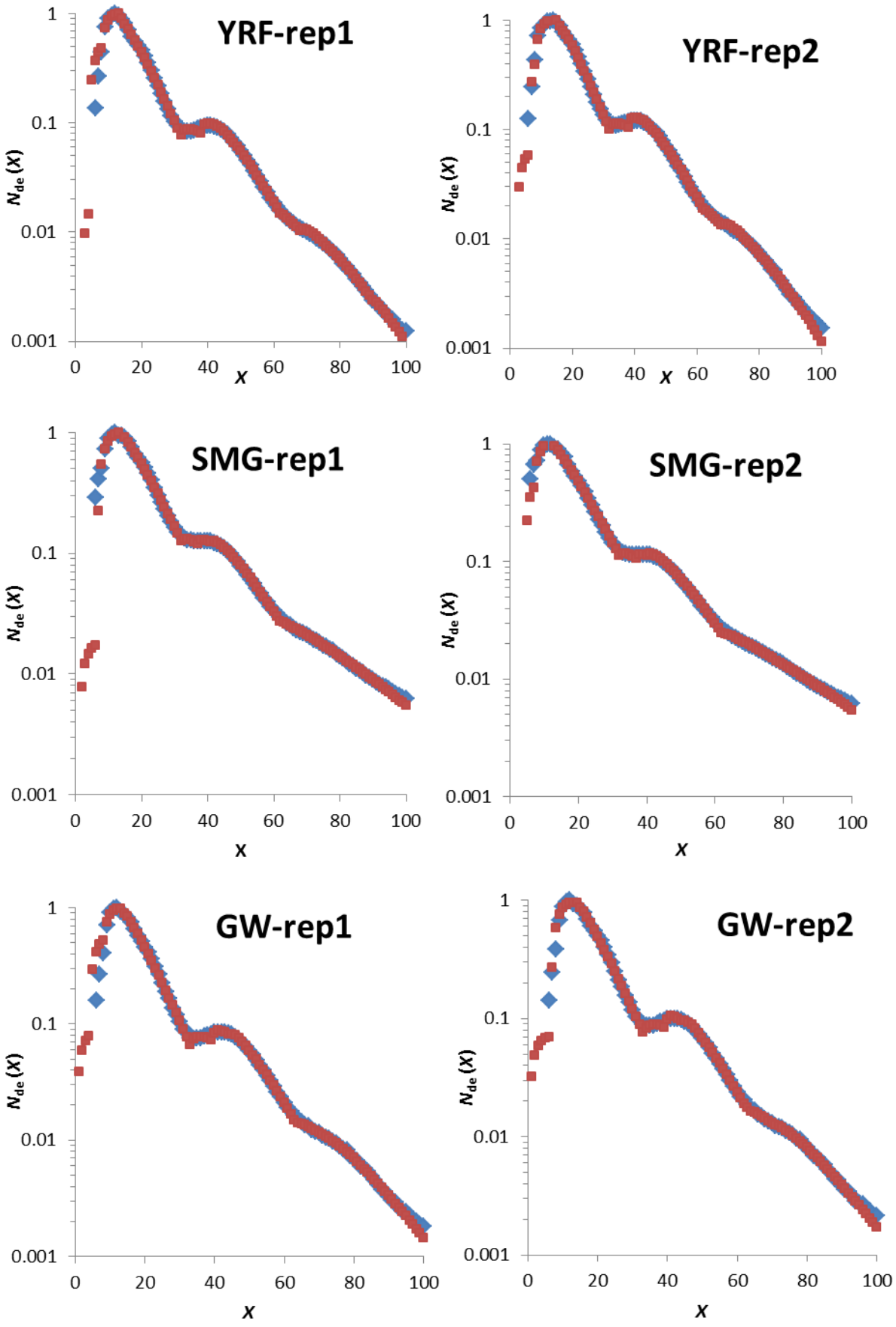
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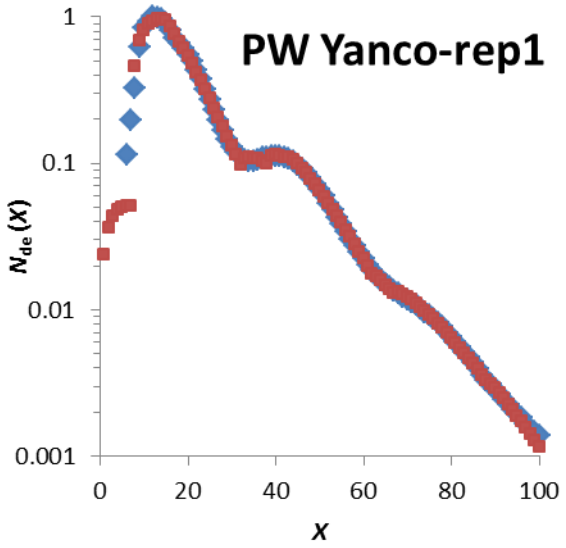
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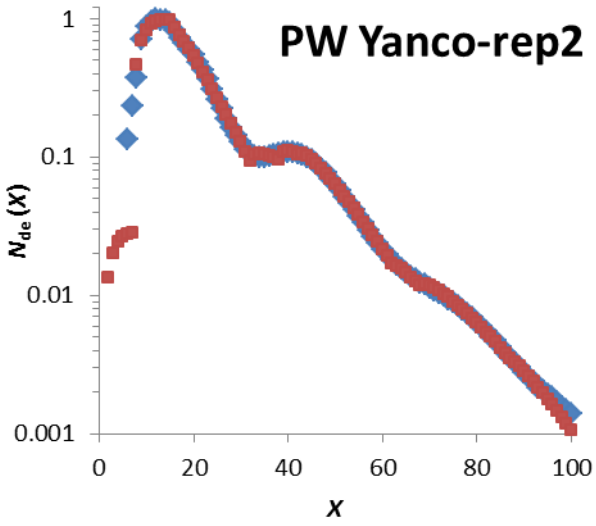
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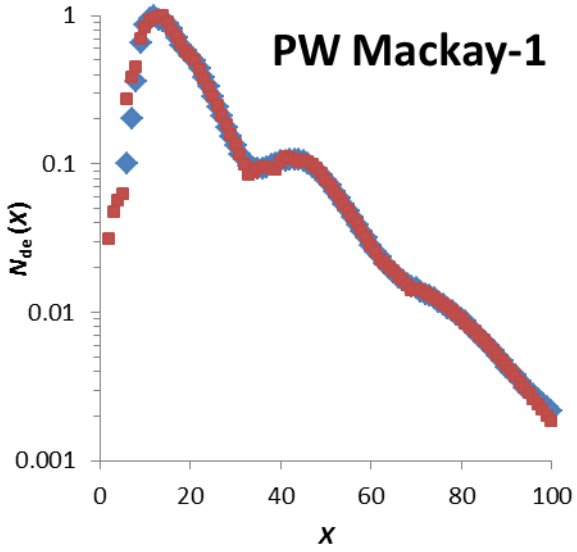
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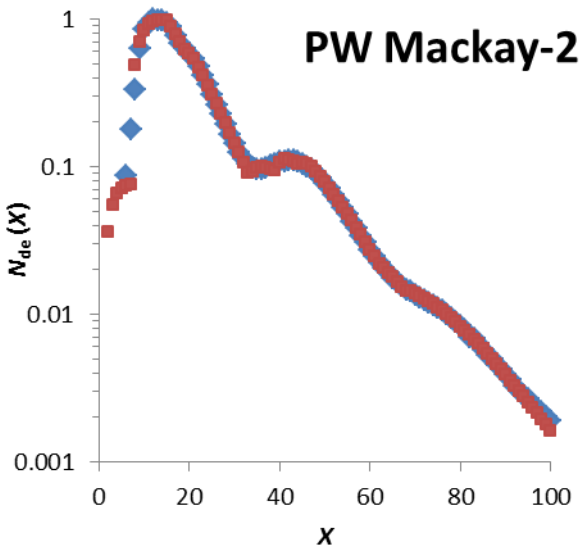
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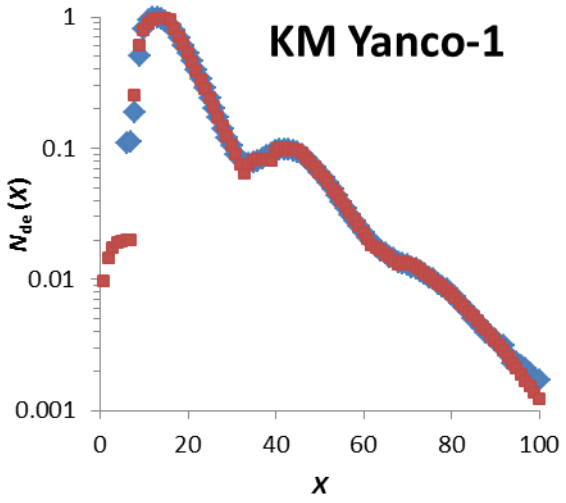
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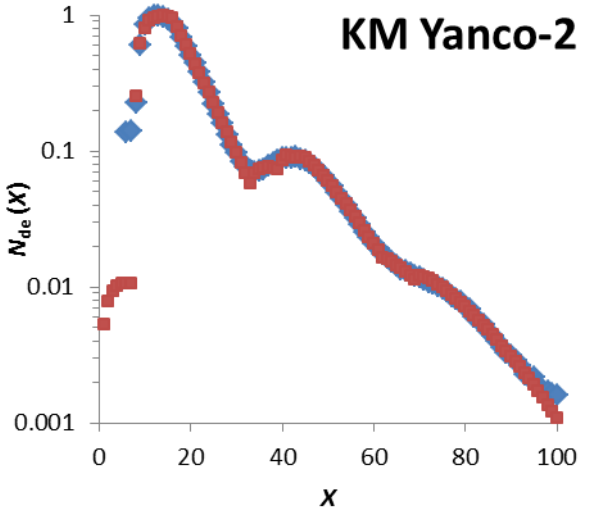
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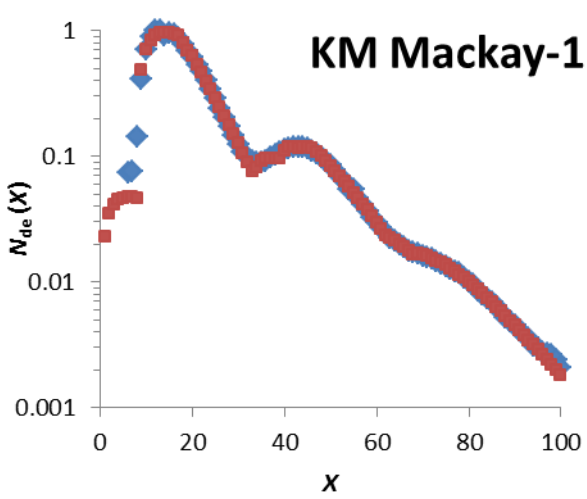
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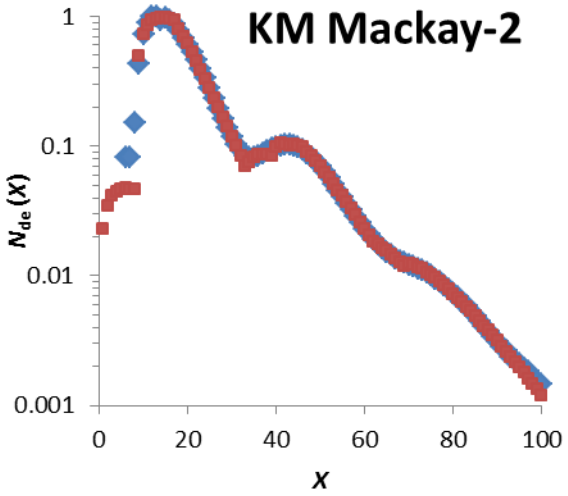
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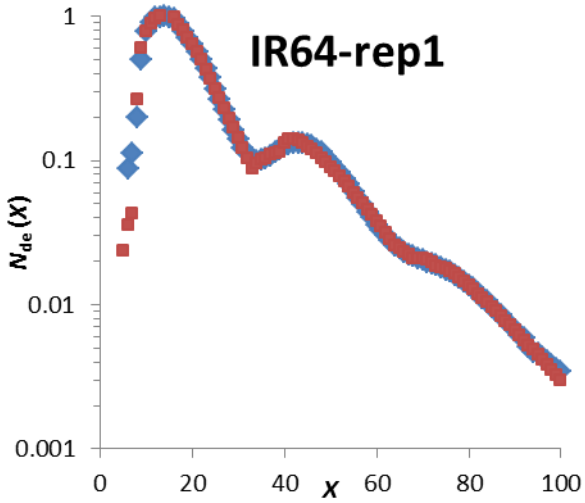
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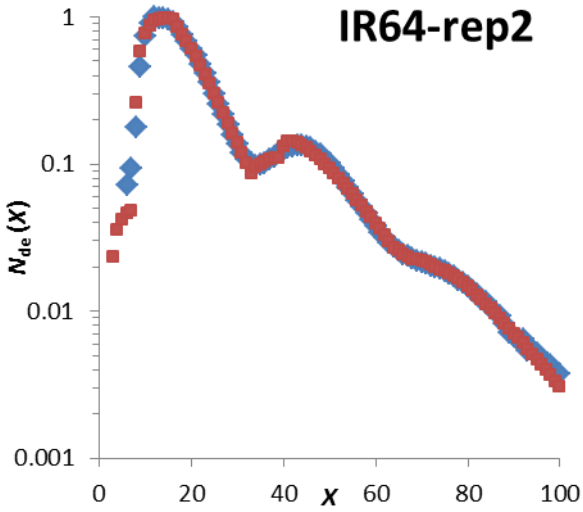
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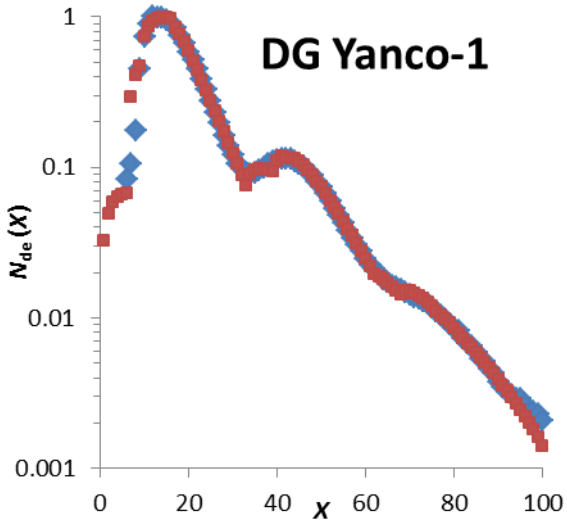
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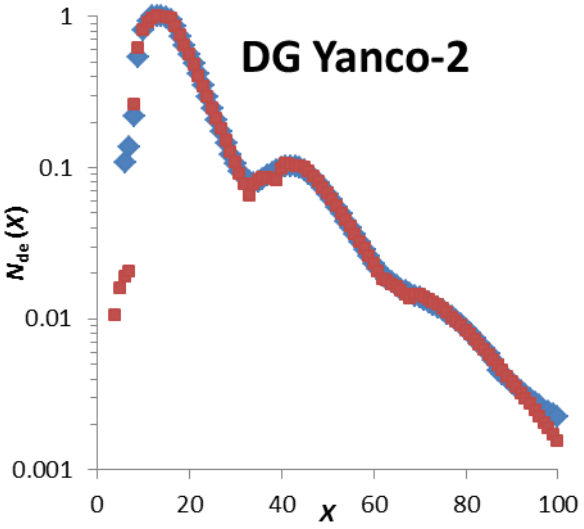
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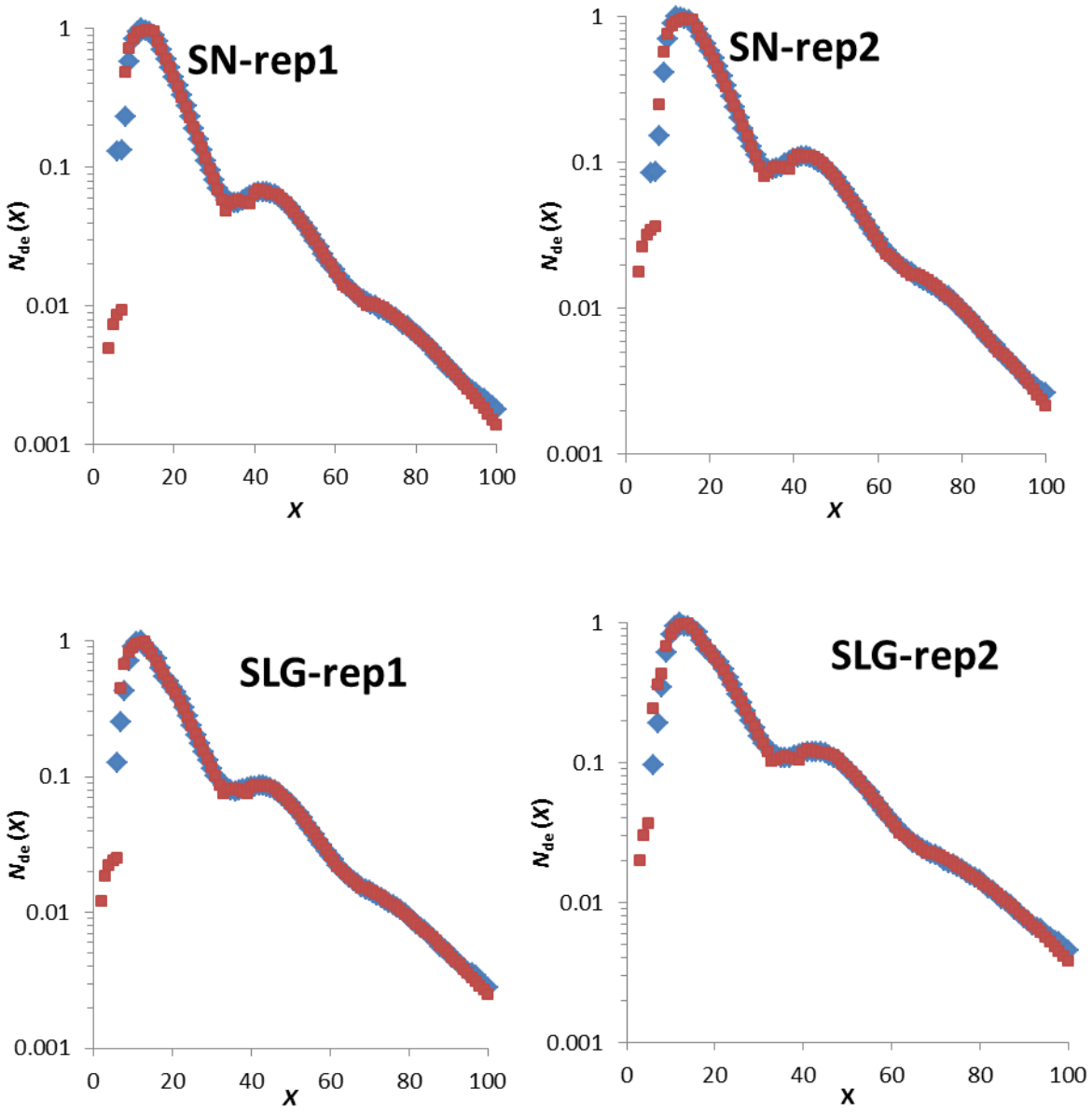

Figure S 4.2 Fitting FACE number CLDs in Wu-Gilbert model.

Table S 4.1 Descriptive sensory analysis attributes and definitions for evaluating cooked rice texture

Phase/Attributes	Definition	Reference	
PHASE I. Place 6-7 grains of rice in mouth . Press tongue over surface and evaluate (No chewing).			
Initial Starchy Coating	The amount of paste-like thickness perceived on the rice. It is present initially and disappears after approximately 3 passes of the tongue.	Jello	0
		Cooked Starch Paste	7
Slickness	Maximum ease of passing tongue over the rice surface. This measurement immediately follows the initial coating measurement and may be short lived.	Whole Wheat Spaghetti	7.5
		Spaghetti	10
Roughness	The amount of particles in the surface	Jello	0
		Potato chips	4
		Cheerios	7
Stickiness to lips	The degree to which the surface of the sample adheres to the lips.	Breadsticks	4
		Pretzel rod	10
Stickiness between grains	Degree to which the samples holds together when first placed in the mouth and separated into individual pieces by the tongue.	Cornbread	4
PHASE II. Place ½ spoon of rice in mouth. Evaluate before or at first bite (No chewing).			
Springiness	The degree to which sample returns to original shape	Cream Cheese	0
		Cheese	3
		Marshmallow	9.5
Cohesiveness	The degree to which sample deforms rather than crumbles, cracks, or breaks	Cheese	5
		Dried fruit	10
		Chewing fruit	15
Hardness	The force to attain a given deformation such as force to compress between molars	Cream Cheese	1
		Egg white	2.5
		Olives	6
PHASE III. Evaluate during chewing			

Uniformity of bite	Degree to which the product changes from start to finish in the bite. If the force necessary to bite through the sample changes during the bite, the product is non-uniform.	Shredded Wheat	3.5
		Cream Cheese	15
Cohesiveness of mass	The degree to which chewed sample (at 10 to 15 chews) holds together in a mass	Carrots	2
		Mushroom	4
		Cheese	9
Moisture absorption	The amount of saliva absorbed by sample during chew down	Popcorn	7.5
		Wheat thins	15
PHASE IV. Evaluate after swallow			
Residule Loose Particles	Amount of particles remaining in the mouth after swallowing	Spaghetti	4.5
		Carrots	10
Toothpack	The degree to which product sticks on the surface of teeth	Carrots	1
		Mushrooms	3
		Cheese slices	9

Table S 4.2 Sensory scores for textural attributes of all rice varieties.

Rice variety	Initial starchy coating	slickiness	roughness	Stickiness to lips	stickiness between grains	Springiness	Cohesiveness	Hardness	Uniformity of bite	Cohesiveness of Mass	Moisture Absorption	Residual loose particles	Toothpack
KN	4.33 ^{a,b}	4.56 ^{b}	2.44 ^{b,c}	13.78 ^{a}	9.72 ^{a}	6.94 ^{a}	9.39 ^{a}	2.50 ^{b}	12.06 ^{a,b}	10.89 ^{a,b}	8.83 ^{a}	4.83 ^{a,b}	10.56 ^{a}
HMN	5.06 ^{a}	5.44 ^{a,b}	1.89 ^{c}	14.00 ^{a}	12.06 ^{a}	4.72 ^{a}	10.06 ^{a,b}	3.06 ^{a,b}	12.83 ^{a}	11.50 ^{a}	9.28 ^{a}	3.89 ^{b}	6.67 ^{b}
KG	3.61 ^{a,b}	6.67 ^{a,b}	3.22 $^{a-c}$	10.33 ^{b,c}	4.72 $^{b-e}$	5.17 ^{a}	7.17 ^{b,c}	3.83 ^{a,b}	10.28 ^{a,b}	8.56 $^{c-e}$	9.94 ^{a}	5.94 ^{a,b}	5.28 $^{c-e}$
SJ	3.78 ^{a,b}	6.56 ^{a,b}	3.11 $^{a-c}$	10.44 ^{b,c}	5.17 $^{b-d}$	5.50 ^{a}	7.78 $^{a-c}$	4.00 ^{a,b}	9.67 ^{a,b}	8.22 $^{c-e}$	9.67 ^{a}	5.67 ^{a,b}	5.22 $^{c-e}$
KDML	3.83 ^{a,b}	6.67 ^{a,b}	3.00 $^{a-c}$	10.72 ^{b,c}	6.00 ^{b,c}	5.72 ^{a}	8.44 $^{a-c}$	3.28 ^{a,b}	10.78 ^{a,b}	9.44 ^{b,c}	9.61 ^{a}	4.83 ^{a,b}	5.50 $^{c-e}$
LG	3.56 ^{a,b}	6.89 ^{a}	3.72 ^{a,b}	9.89 ^{c}	4.61 $^{b-e}$	5.67 ^{a}	7.50 $^{a-c}$	3.83 ^{a,b}	10.39 ^{a,b}	9.17 ^{b,c}	10.33 ^{a}	5.44 ^{a,b}	5.61 $^{b-d}$
YRF	4.39 ^{a,b}	7.06 ^{a}	2.39 ^{b,c}	10.78 ^{b,c}	6.06 ^{b,c}	5.67 ^{a}	8.22 $^{a-c}$	3.44 ^{a,b}	11.44 ^{a,b}	9.44 ^{b,c}	9.56 ^{a}	5.39 ^{a,b}	5.44 $^{c-e}$
SMG	3.56 ^{a,b}	6.94 ^{a}	2.39 ^{b,c}	11.11 ^{b}	6.28 ^{b}	6.22 ^{a}	8.33 $^{a-c}$	3.67 ^{a,b}	10.39 ^{a,b}	9.50 ^{b,c}	9.56 ^{a}	5.56 ^{a,b}	5.94 ^{b,c}
GW	3.33 ^{a,b}	6.94 ^{a}	2.83 $^{a-c}$	10.28 ^{b,c}	5.00 $^{b-e}$	5.94 ^{a}	7.44 $^{a-c}$	4.11 ^{a,b}	10.11 ^{a,b}	8.50 $^{c-e}$	9.61 ^{a}	5.28 ^{a,b}	5.39 $^{c-e}$
PW-Yanco	3.72 ^{a,b}	6.50 ^{a,b}	3.39 $^{a-c}$	10.94 ^{b,c}	4.67 $^{b-e}$	5.33 ^{a}	7.89 $^{a-c}$	4.06 ^{a,b}	9.33 ^{a,b}	9.22 ^{b,c}	9.61 ^{a}	5.61 ^{a,b}	5.33 $^{c-e}$
PW-Mackay	3.67 ^{a,b}	6.83 ^{a}	3.11 $^{a-c}$	10.22 ^{b,c}	5.00 $^{b-e}$	5.67 ^{a}	8.00 $^{a-c}$	3.39 ^{a,b}	9.83 ^{a,b}	8.44 $^{c-e}$	9.50 ^{a}	5.61 ^{a,b}	4.83 ^{d,e}
KM-Yanco	3.56 ^{a,b}	6.28 ^{a,b}	3.00 $^{a-c}$	10.28 ^{b,c}	5.33 $^{b-d}$	5.44 ^{a}	7.06 ^{b,c}	3.44 ^{a,b}	9.67 ^{a,b}	8.56 $^{c-e}$	9.22 ^{a}	5.28 ^{a,b}	5.11 $^{c-e}$
KM-Mackay	3.78 ^{a,b}	6.67 ^{a,b}	2.89 $^{a-c}$	11.00 ^{b,c}	5.67 $^{b-d}$	5.78 ^{a}	7.94 $^{a-c}$	3.28 ^{a,b}	9.94 ^{a,b}	8.94 ^{c}	9.28 ^{a}	5.33 ^{a,b}	5.28 $^{c-e}$
IR64	3.06 ^{a,b}	7.22 ^{a}	3.22 $^{a-c}$	6.72 ^{d}	4.78 $^{b-e}$	5.17 ^{a}	7.67 $^{a-c}$	4.67 ^{a}	9.00 ^{a,b}	9.22 ^{b,c}	9.44 ^{a}	5.39 ^{a,b}	5.06 $^{c-e}$
DG-Yanco	3.06 ^{a,b}	6.56 ^{a,b}	3.22 $^{a-c}$	6.44 ^{d}	3.56 $^{c-e}$	5.61 ^{a}	7.39 $^{a-c}$	4.22 ^{a,b}	9.28 ^{a,b}	8.06 $^{c-e}$	9.39 ^{a}	5.22 ^{a,b}	4.72 ^{d,e}
DG-Mackay	3.28 ^{a,b}	6.50 ^{a,b}	2.78 $^{a-c}$	4.72 ^{e}	4.78 $^{b-e}$	5.94 ^{a}	7.72 $^{a-c}$	4.00 ^{a,b}	10.17 ^{a,b}	8.78 ^{c,d}	9.33 ^{a}	5.11 ^{a,b}	5.50 $^{c-e}$
SN	2.28 ^{b}	5.56 ^{a,b}	4.28 ^{a}	2.83 ^{f}	2.50 ^{e}	4.72 ^{a}	6.50 ^{c}	4.61 ^{a}	8.39 ^{b}	7.11 ^{d,e}	9.39 ^{a}	6.44 ^{a}	5.11 $^{c-e}$
SLG	2.75 ^{b}	6.06 ^{a,b}	3.83 ^{a}	3.25 ^{f}	3.58 $^{d-e}$	5.11 ^{a}	6.72 ^{c}	4.58 ^{a}	9.11 ^{b}	7.08 ^{e}	9.89 ^{a}	6.33 ^{a}	4.67 ^{e}

Values with different letters in the same column are significantly different with $p < 0.05$

Chapter 5 Conclusions and recommendations

5.1. Conclusions

Fig. 5.1 summarizes the cause and mechanism of the hardness and stickiness of cooked rice grains. Briefly, rice with higher amylose content always tends to have harder and less sticky texture after rice cooking. In detail, rice with smaller amylose size and higher proportion of short amylose chains tends to have a harder texture after cooking. The likely mechanism is that these amylose molecules may entangle and/or co-crystallize with amylopectin chains in the crystalline lamellae, thereby causing limited starch swelling during rice cooking and a harder texture (Vandeputte, Derycke, Geeroms & Delcour, 2003). The limited swelling could also limit starch leaching out starch granules and rice kernels during rice cooking, which potentially affects the stickiness between cooked rice grains. For stickiness between cooked rice grains, in the surface layer where the leachate is located, if there are less amylopectin molecules with smaller amylopectin molecular size and lower proportion of short amylopectin chains, this will cause less bonding sites when the TPA probe descends and touches the rice kernels. Correspondingly, these amylopectin molecules can also have weaker interaction between themselves, thereby causing lower viscosity resistance during the TPA debonding process and a less sticky texture.

The overall objectives of this thesis are to explore the molecular mechanisms for the two most important textural attributes, i.e. hardness and stickiness of cooked rice grains; and to develop an improved instrumental evaluation method to overcome the disadvantages of current TPA method. By conducting the studies in this thesis, we have attempted to give answers to the three scientific questions proposed in the section of **1.5**, which also associate to the overall objectives of this thesis.

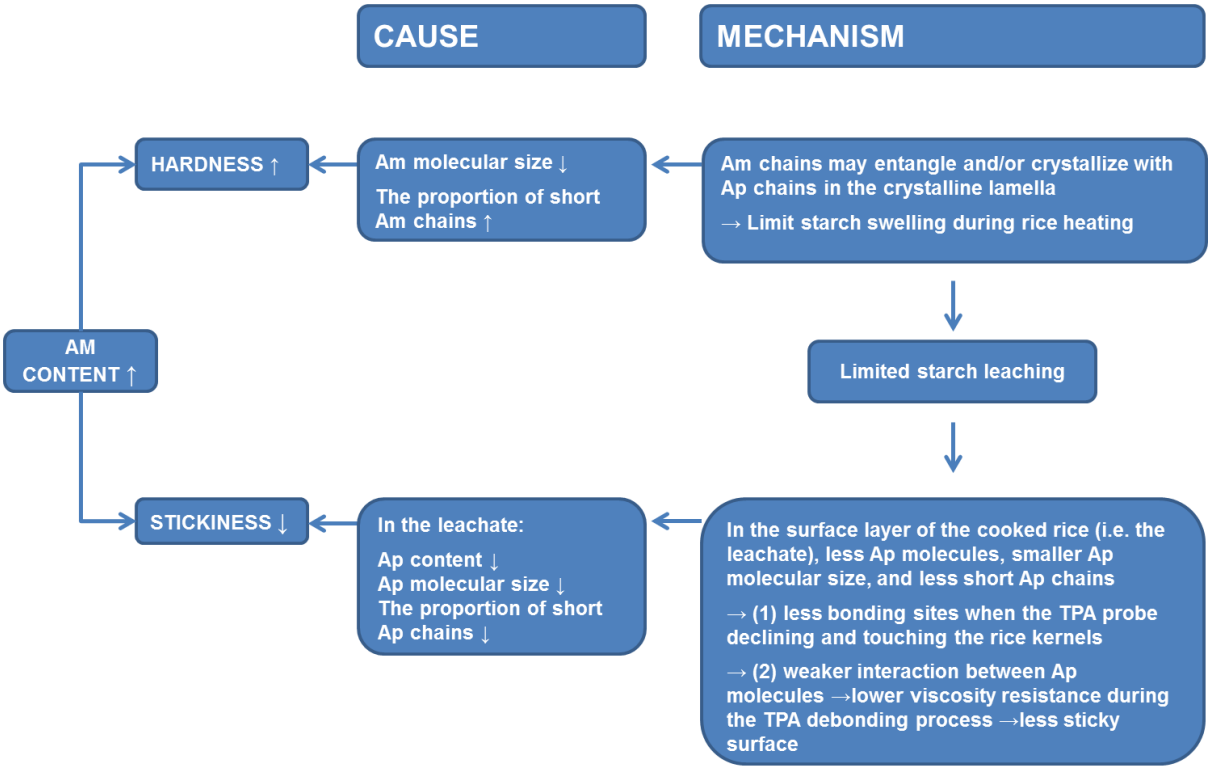

Figure 5.1 Summarizing diagram for the mechanism of the hardness and stickiness of cooked rice.

Question 1: what is the structural basis for the hardness of cooked rice?

Chapter 2 gives a new perspective on the relationship between the fine structure of amylose and amylopectin and the texture of cooked rice. The correlations found here support past studies that have found the amylose content to be important for the texture of cooked rice. Our study also shows, for the first time, that the whole amylose molecular size and the proportion of amylose branches ranging from 1000 to 2000 DP have significant effects on the hardness of cooked rice. A smaller amylose molecular size and a higher proportion of amylose branches with DP from 1000 to 2000 were found in the varieties with intermediate and high amylose, and these also led to an increase in hardness. How these structural features affect amylose leaching during cooking, and/or the degree of starch granule swelling during heating, may help explain the mechanism for this increase in hardness. Additionally, the amylopectin content and short chains of amylopectin are significantly and positively correlated with the stickiness of cooked rice samples with a wide range of amylose content. This study provides valuable information for further research to progress our understanding of (i) the relationship between the fine structure of starch and the sensory properties of rice, and (ii) the genetic regulation of the starch biosynthetic pathway.

Question 2: what is the structural basis for the stickiness between cooked rice grains?

This study reveals that stickiness between cooked rice grains is determined by the total amount, molecular size and chain structure (CLD) of leached amylopectin. We present the first unified molecular-based mechanistic description of the causes of these important sensory properties, using the results in this study and previous findings by ourselves (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a, b) and others (Cameron & Wang, 2005; Ong & Blanshard, 1995b; Patindol, Gu & Wang, 2010). Starches with certain structural features can leach from rice kernels during cooking and attach on the surface of the cooked rice grains. The molecular size of leached amylopectin is about 30 times smaller than that of native amylopectin, while that of leached amylose is about 5 times smaller than that of grain amylose. Leached amylopectin has a similar CLD to that in the grain, while the leached amylose branches have smaller chain lengths, mainly between DP 100 – 1000. The postulated mechanism for stickiness between cooked rice grains and the probe is that an increase of the amount of amylopectin, the proportion of short amylopectin chains, and amylopectin molecular size in the leachate all create a greater opportunity for bonding and molecular interaction, causing more force to be needed to make the grains and probe come apart, i.e. a higher stickiness value. This result could also give insight into the molecular mechanism why parboiled rice always displays a reduced stickiness compared to that of nonparboiled rice, which has been interpreted as the result of reduced swelling and limited starch leaching ability.

An underlying origin of the stickiness differences between rice cultivars is the amylose content in the whole grain starch. With increasing amylose content, the total amount of leached materials, the amylopectin content in the leachate, and the molecular size and the proportion of short branches of leached amylopectin, all decrease, leading to a lower stickiness. However, amylose content is not the sole determinant. In some cases, amylose content is similar but the hardness (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a) and/or stickiness (Ayabe, Kasai, Ohishi & Hatae, 2009) still vary significantly. This is because of the effects of other structural features. One such is amylose chain-length distributions. Our previous finding points out that high-amylose rice tends to have higher proportions of short amylose chains (Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a). Whether this is a characteristic of all high-amylose rices could provide insight into their functional differences. Another determining structural feature is the interaction between

amylose and amylopectin molecules (the location of amylose) in native starch granules. The location of amylose in native starch granules is not completely understood, but it is often thought that amylose molecules are present in an amorphous conformation (Lopez-Rubio, Flanagan, Shrestha, Gidley & Gilbert, 2008; Morrison, Law & Snape, 1993); further, there are suggestions that amylose is spread among amylopectin crystallites (Jane, Xu, Radosavljevic & Seib, 1992; Li, Prakash, Nicholson, Fitzgerald & Gilbert, 2016a), and may co-crystallize with amylopectin chains.

Question 3: How to overcome disadvantages of the current TPA method and develop an improved instrumental method for the evaluation of the texture of cooked rice?

Chapter 4 shows that sensory descriptive analysis and two instrumental methods for evaluating the hardness and stickiness of cooked rice are significantly correlated. Specifically, the rheological quantity K^* positively correlates with hardness tested by TPA and panellists while tan δ shows positive correlations with stickiness by TPA and stickiness to lips by panellists. On the other hand, K^* represents the elasticity of cooked rice while tan δ represents viscous characteristics, indicating it is also mechanistically meaningful as well as preferable to use dynamic rheological testing as an alternative to TPA (although a disadvantage is the cost of the instruments involved). Further, the present novel instrumental method overcomes certain limitations of conventional TPA measurement: good reproducibility and easy operability. Additionally, it is carried out on bulk of cooked white rice, instead of a couple of rice kernels, which is closer to human perceptions.

The differences in fine structures of amylopectin and amylose can be seen to be causally controlling the textural differences between rice samples. Amylose content and the proportion of long amylopectin branches ($70 \leq X < 100$) are positively correlated with K^* tested by dynamic rheology and hardness tested by TPA and panellists, indicating that rices with higher content of both amylose and long amylopectin branches might be resistant to swelling during cooking, correspondingly, causing a more elastic and less viscous texture. This can be ascribed to entanglement of these longer chains slowing down the swelling process, equivalent to well-known mechanical effects with synthetic polymers.

In conclusion, the studies in this thesis suggest potential new procedures for the rice industry and rice breeders. For example, by quantifying the components and the molecular structure of leached starch, rice breeders could choose lines which optimize the texture of cultivars. A

cultivar which leaches more amylopectin with more short amylopectin chains and bigger molecular size would be stickier after cooking, which could be desirable for sushi. On the other hand, a cultivar which leaches more amylose should be less sticky but have a harder texture. This molecular structural mechanism provides a new tool for rice breeders to select cultivars with desirable palatability.

5.2. Recommendations

This thesis has demonstrated the relations between molecular structure, textural property and sensory perceptions of cooked rice grains; an improved instrumental method to evaluate the texture of cooked rice is also introduced. It gives improved understandings of the texture of cooked rice grains from molecular, instrumental and sensory levels, it also provides information for further research to progress our understandings of:

I. The specific location of amylose molecules within starch granules. From our studies, the amylose molecules play a significant role on hardness and stickiness of cooked rice grains. However, it is still unclear how amylose interacts with amylopectin in the granule. Some structural biologists believe that long amylose-like chains are present in more or less regularly spaced amorphous cavities within the granules (Blanshard, 1987). Others have proposed that amylose can also infiltrate the amylopectin crystalline structure, because small-angle X-ray scattering experiments have shown that starches with increasing amylose content display changes in the ratio of the amorphous to crystalline lamellae within the unit amylopectin cluster (Jenkins & Donald, 1995). However, this particular study compared starches from different botanical sources, some of which are known to differ in their amylopectin synthesis machinery (Ball, van de Wal & Visser, 1998). Thus, in the future work, by using starches from the same botanical origin and with similar amylopectin CLD, the effect of amylose content on the crystalline, lamellar structures of amylopectin should be explored.

II. The optimization of the reference samples for sensory training. From sensory results in Chapter 4, we can see that when rices with a wide range of amylose content are used, rice varieties can be classified into 3 different groups: waxy rice, high-amylose rice and low-amylose rice. However, for the low-amylose rices, it is hard to make the classification. This is probably because in the sensory training session we used the very general references which are always used for a wide range of food products'

sensory training, making the differences of a specific attribute between low-amylose rice samples difficult to discriminate. So, in the future work, a set of specific references for the textural evaluation of cooked rice should be developed.

III. The effect of mastication and saliva on the rheological properties of cooked rice. Food texture is regarded as a multidimensional sensory property that is influenced by the food's structure, rheology, and even surface properties. Oral processing has been suggested to split into the following 6 stages: (i) first bite, (ii) comminution, (iii) granulation, (iv) bolus formation, (v) swallow and (vi) residue (Stokes, Boehm & Baier, 2013). However, in current study, no matter whether using the TPA or the rheological test, both methods evaluate the textural attributes in terms of cooked rice grains without chewing; this does not reveal information about how mastication affects the textural property of cooked rice, therefore, it would be useful to include mastication and/or saliva into the system for evaluation, to see how the rheological property changes with these.

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